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**Effects of temperature and humidity on the mortality of the tick Dermacentor reticulatus in the UK**

# Effects of temperature and humidity on the mortality of the tick *Dermacentor reticulatus* in the UK

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# Abstract

The disease canine babesiosis, transmitted by the tick *Dermacentor reticulatus*, has recently been found for the first time in the UK. This disease outbreak highlighted our lack of knowledge of the distribution and abundance of this tick species in the UK, which is believed to be expanding under the influence of climate change. However, given our limited understanding of the influence of microclimatic variables on its activity, it is difficult to predict its future spread to manage any potential risks. The research presented in this thesis aimed to quantify the effects of temperature and relative humidity on the mortality of *D. reticulatus*.

Adult ticks were collected between September 2019 and February 2020 from 4 locations in the UK: West Wales, Essex, North Devon, and South Devon. Different concentrations of potassium hydroxide were used to create a range of humidities inside sealed desiccator jars, each one kept in incubators at temperatures of 4°C, 15°C or 30°C. Five relative humidities were tested: 20%, 40%, 60%, 80% and 95%. Each desiccator contained three tubes of five ticks which were checked every 2 or 3 days for mortality over a period of 9 weeks (63 days).

Mortality increased significantly over time. The data show that tick survival rate was lower when the temperature was higher, the humidity was lower, and the longer the ticks had been exposed to these conditions in the desiccator. Integrating temperature and humidity to calculate a measure of saturation deficit showed that there was no effect on tick survival rate until day 20 and that where the saturation deficit value was lower, indicating a high air moisture content, the survival rate increased.

This study suggests that *D. reticulatus* is able to survive at relatively low temperatures, lower than those preferred by other species of ticks such as *I. ricinus* and *I. hexagonus*, and that an environmental humidity exceeding 80% RH is required for *D. reticulatus* ticks to prevent water loss via evaporation.

These results have implications for predictions about how *D. reticulatus* populations and tick-borne disease will be affected by climate change in the UK. It may be that *D. reticulatus* will spread northwards in the UK seeking cooler temperatures, perhaps more rapidly than other UK species such as *I. hexagonus* and *I. ricinus* which tolerate warmer environments. However, this will also be strongly affected by changes in precipitation. Higher rainfall will allow *D. reticulatus* survival even at elevated average temperature. As a result, pathogens transmitted by *D. reticulatus* will become more abundant and more widely established in areas of the UK they are not usually found in. Higher temperatures may also cause more successful incubation of pathogens with *D. reticulatus*, leading to a higher risk of disease.

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Lastly, I would like to thank my parents, friends and flatmates for their support and assistance throughout the year.

# Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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# CHAPTER 1

# Introduction: Ticks and tick-borne disease

## 1.1 Systematics of ticks

The first scientific description of a tick is thought to have been made by Linnaeus in 1746 (Barker and Murrell, 2004). Today it is considered that there are around 899 species of tick within the subclass Acari and suborder Ixodida, separated into three extant families (Barker and Murrell, 2004): the Ixodidae (hard ticks), the Nuttalliellidae, comprised of a single species *Nuttalliella namaqua,* and the Argasidae (soft ticks) (Camicas *et al.,* 1998).

*Nuttalliella namaqua* is thought to be a representative of the last common ancestral tick lineage as it bears characteristics of both the Ixodidae and the Argasidae in addition to some derived features. It feeds on reptiles and is found only in southern Africa, which suggests that this region may have been where ticks originated (Mans *et al.,* 2011).

The Ixodidae have a wide global distribution (Grist, 1992). The family is divided into two groups based on differing morphological traits and, more recently, molecular methods (Charrier *et al.,* 2019). The Metastriata is made up of ~450 species within the genera including *Amblyomma, Rhipicephlus (Boophilus), Dermacentor* and *Hyalomma* (Barker and Murrell, 2004). The Prostriata is comprised of ~250 species within a single genus, *Ixodes* (Charrier *et al.,* 2019). Argasidae, the soft ticks, are composed of ~200 species within 5 currently acknowledged genera; *Antricola, Argas, Nothoaspis, Ornithodoros* and *Otobius.* However, the taxonomy of Argasidae is a subject of ongoing debate among taxonomists (Guglielmone *et al.,* 2010). Argasidae are usually found more commonly in areas in which the climate is warm and relatively dry. Consequently, they are not commonly found in abundance in the UK, but instead are widely distributed in the Americas, Africa, and Eurasia.

## 1.2 The Ixodida: morphology and life cycle

There are multiple anatomical differences between Argasidae and Ixodidae ticks. Soft ticks lack the defining feature of the Ixodidae, the hard sclerotised dorsal plate known as the scutum or conscutum, and instead their dorsal side has a tough, waterproof integument with a mammillated texture. Generally, all nymphal and adult ticks have 4 pairs of legs, specialised mouthparts and bodies comprised of two parts, the posterior idiosoma onto which attach the legs, and the anterior gnathosoma (capitulum) which includes the specialised mouthparts (Needham and Teel, 1991; Sonenshine and Roe, 2014). These mouthparts, made up of the chelicerae, palps, and a hypostome, make ticks distinct among the Acari and are adaptations to their obligate hematophagous behaviour. They are ectoparasites of mammals, reptiles, birds and amphibians and feed on the blood of their hosts by using their paired chelicerae to pierce the dermis and tear the capillaries at the skin surface (Kaufman, 1989; Sonenshine and Roe, 2014).

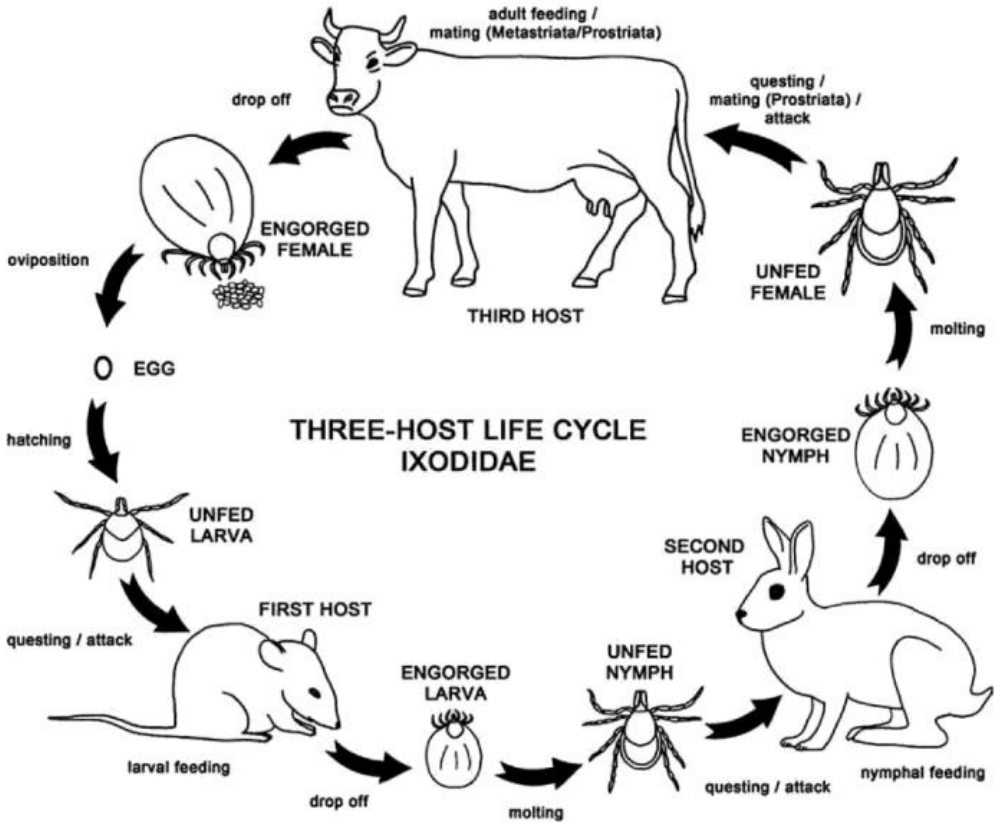


Fig 1.1 The three-host life cycle of female Ixodidae ticks (Sonenshine and Roe, 2014).

Most ticks go through 4 life cycle stages: egg, larva, nymph, and adult. The larvae have 6 legs and the nymphal stages have 8. Most species have evolved to have a three-host life cycle (Fig 1.1), whereby each instar feeds on a different host before dropping off to moult into the next stage. One and two-host life cycles are less common; moulting from larva to nymph to adult occurs on the same host in one-host cycles, and the adult will locate a new host in two-host cycles. Ixodidae have a single nymphal instar, whereas Argasidae can have several (Oliver, 1989; Sonenshine and Roe, 2014). Ixodidae females have a small scutum, allowing for expansion of the idiosoma as they blood-feed over the course of several days before dropping off the host, laying several thousand eggs in the environment then dying; mating having usually occurred on the host (Sonenshine and Roe, 2014; Estrada-Peña, 2015). In contrast, Argasidae females feed repeatedly and lay between 200-500 eggs between each blood-meal, although this number varies depending upon tick species, size of the female, and the amount of blood ingested. In Argasidae, mating occurs in the environment (Feldman-Muhsam and Borut, 1971; Hoogstraal, 1985; Oliver, 1989). The method used to digest the blood of their hosts also differs. Ixodidae secrete a large proportion of the surplus water from the blood-meal back into the host via the salivary glands. In contrast, Argasidae use the ultrafiltration organ on their coxae, called coxal organs (Estrada-Peña, 2015). Male ticks of most genera usually feed just enough for their reproductive organs to fully develop, although some, such as *Ixodes* spp.*,* moult from their nymphal form with already active reproductive organs therefore are not required to feed at all. Male ticks remain on the host to mate, which they achieve externally by transferring a spermatheca to the female (Oliver *et al*., 1974; Sampieri *et al.,* 2016).

Most tick species exhibit endophilic behaviour whereby each life cycle stage shelters in nests, burrows, foliage, or leaf litter until optimum weather conditions permits movement to the top of vegetation to wait for a new host, an activity called questing. The tick latches onto a new host using its front legs and then moves to a suitable location to feed. Questing ticks are attracted to host stimuli, such as body heat, $NH_3$ and $CO_2$. These cues are detected using the Haller's organ, a highly sensitive pit, packed with chemo-, temperature, and other receptors, positioned on the first pair of legs (Sonenshine and Mather, 1994; Sonenshine and Roe, 2014). Most tick species adopt this sit-and-wait strategy for host location. Only two genera, *Amblyomma* and *Hyalomma,* exhibit exophilic behaviour by actively hunting their hosts and moving towards them (Uspensky, 2002; McGarry, 2011). Host-seeking behaviour is dependent upon environmental factors such as changing photoperiod, temperature, humidity, and incident solar energy (Sonenshine and Mather,

1994). During questing behaviour, ticks lose water quickly so must rehydrate by descending the vegetation into the litter where humidity levels are higher and water vapour can be absorbed from the atmosphere (Perret *et al.,* 2003).

As soon as humidity conditions are appropriate and/or ticks have hydrated appropriately, questing activity can resume (Estrada-Peña, 2015). When off the host, ticks are in danger of desiccation, predation, starvation, and temperature change (Randolph, 2004a). Questing usually occurs in seasonal patterns, dictated by the climatic conditions required by each tick species and the presence of their specific hosts. Awareness of these patterns is necessary when attempting to assess the risk of pathogen transmission for both humans and animals and for knowing when to apply control measures with greatest effect (Sonenshine and Mather, 1994).

Tick development may cease during its life cycle due to diapause. This is when development ceases seasonally, orchestrated by hormonally controlled mechanisms which allow it to avoid imminent unfavourable conditions. This ensures that oviposition and moulting will occur at optimum summer temperatures. The primary stimulus for inducing diapause within ticks is thought to be photoperiod and temperature (Gray *et al.,* 2016).

## 1.3 How ticks transmit disease

Ticks are important vectors of a wide range of pathogens globally, due to their high fecundity, environmental resilience, and extensive range of possible hosts, so are of medical and veterinary significance. Among the blood-feeding arthropods, ticks transmit the greatest range of pathogens to host animals (Needham and Teel, 1991); it is thought that an estimated 10% of species are able to transmit pathogens (Jongejan and Uilenberg, 2004). Pathogens are acquired in the blood-meal when the tick feeds from an infected host.

Ticks act as a primary reservoir host and transmission from tick to host occurs primarily when the nymph or adult feeds again. Larvae are usually unlikely to carry these pathogens as they have not previously fed and transovarial transmission from the female to the larvae via the eggs is rare. After acquiring a pathogen in an initial blood-meal, the pathogen will usually be retained throughout all subsequent life cycle stages, with trans-stadial transmission (Bremer *et al.,* 2005).

Tick saliva, secreted from the salivary glands, has many functions including countering host responses through various proteins and inhibitors, maintaining host attachment and control of tick homeostasis, but has a further role in intensifying pathogen transmission between individual ticks on the host via the subcutaneous tissue (Nuttall, 2019). This is called salivary assisted transmission (SAT) whereby transmission is not only to the host via the bite, but to uninfected ticks feeding in a nearby location via saliva (Šimo *et al.,* 2017). This is exacerbated through the gregarious feeding behaviour of ticks on hosts through aggregation-attachment pheromones (Petney and Bull, 1981); minimization of space between feeding ticks facilitates SAT (Randolph *et al.,* 1999; Nuttall, 2019). Additionally, there is evidence that proteins produced within the saliva of feeding ticks increase the likelihood of transmission of some tick-borne viruses (Kubes *et al.,* 1994; Hovius, 2009; Marchal *et al.,* 2010).

## 1.4 Ticks in the UK

There are over 20 native species of tick found in the UK that feed on mammals, reptiles, or birds (Medlock *et al.,* 2018). The majority of these are hard ticks, the most common being *Ixodes ricinus, Ixodes hexagonus* and *Ixodes canisuga* (Abdullah *et al.,* 2016). Other tick species reported include *Dermacentor reticulatus, Ixodes frontalis, Haemaphysalis punctata, Hyalomma marginatum* and *Ixodes arboricola,* which are either less common in the UK or introduced through movement of animals across borders (Jameson and Medlock, 2011). *Ixodes ricinus,* the sheep/deer tick, is the most numerous in the UK and is considered one of the most important threats to public and animal health due to being the primary vector of a variety of pathogens, including *Babesia divergens* causing babesiosis, *Anaplasma* spp. causing tick borne fever, *Borrelia burgdorferi* s.l. causing Lyme borreliosis and Louping ill virus (Medlock *et al.,* 2018).

*Ixodes ricinus,* also known as the sheep tick, is widespread in deciduous and mixed woodland, moorland, grassland and heathland in the British Isles where the required microclimatic conditions are also met. *Ixodes ricinus* is a three-host tick, with each instar feeding on a separate host; in the UK immature stages usually feed on smaller mammals and birds and adult ticks parasitise livestock and deer, depending upon the habitat in which the tick is established (Pietzsch *et al.,* 2005). The primary hosts of *I. ricinus* are livestock, domestic animals such as dogs, humans, small mammals, seabirds, bats and tree-hole and cliff-nesting birds (Jameson and Medlock, 2011).

Reproduction occurs on the host, after which females drop off the host into vegetation to lay eggs. *Ixodes ricinus* lay over 2,000 eggs in a period of up to 6 weeks (MacLeod, 1935). Males and females differ in size and weight, length of the idiosoma and differences in the scutum (Dusbábek, 1996). When unfed, males are between 2.5 to 3mm in length and females are larger between 3 to 4mm, although they can exceed 1cm in length when engorged. *Ixodes ricinus* is a dark brown/red colour (Walker, 2018a). *Ixodes ricinus* is known to transmit a multitude of pathogens; those causing babesiosis, Lyme borreliosis, louping ill, tick-borne encephalitis, and anaplasmosis are known to be transmitted by *I. ricinus* in the UK (Lejal *et al.,* 2019).

*Ixodes hexagonus* is a three-host tick, widespread across Europe, North Africa, and east to Tajikistan (Walker, 2018b). It is thought that in the UK, populations are mostly established in the south east and are rarely reported in the north (Abdullah *et al.,* 2016). It is difficult to state the exact distribution of *I. hexagonus* as it is a primarily nest-dwelling species and ticks outside of nests are seldom encountered, thus the actual distribution and abundance of this species is probably greater than present predictions (Arthur, 1951). The primary host of *I. hexagonus* is hedgehogs, mostly northern white-breasted hedgehogs (*Erinaceus roumanicus*) and European hedgehogs (*Erinaceus europaeus*). *Ixodes hexagonus* is considered to be a red/brown colour and females have a distinct dorsal scutum which is hexagonal shaped. This species is considered somewhat larger than others. Engorged females can exceed 8mm in length and when unfed are between 3.5 to 4mm; females appear lighter in appearance than males. Males are between 3.5 to 3.8mm when unfed (Walker, 2018b). Females spend between 19 and 25 days laying around 1000 to 1500 eggs before dying, and larvae hatch after up to 59 days (Arthur, 1951).

*Ixodes canisuga* is three-host tick common in central and western Europe, including the UK, and is often misidentified as *I. hexagonus* (Hornok *et al.,* 2017). It is thought that *I. canisuga* is more northern in its distribution in the UK, whereas *I. hexagonus* is more commonly found in the south (Harris and Thompson, 1978). This species is often found on domestic animals, especially dogs, in the UK (Ogden *et al.,* 2000), but has also been observed parasitizing foxes (Harris and Thompson, 1978).

## 1.5 *Dermacentor reticulatus*

### 1.5.1 Morphology and life cycle

*Dermacentor reticulatus,* first described by Fabricius in 1974, is also known as the ornate cow tick, marsh tick or winter tick (Medlock *et al.,* 2018). It is found on every continent except Australia and is considerably larger than most other ticks found in the UK. A fully engorged female can reach 1cm in length and unfed ticks are between 3.8-4.2 mm in length. Unfed males are slightly larger between 4.2-4.8mm and nymphs and larvae are smaller in size, between 1.4-1.8mm and 0.5mm, respectively (Földvári *et al.,* 2016).

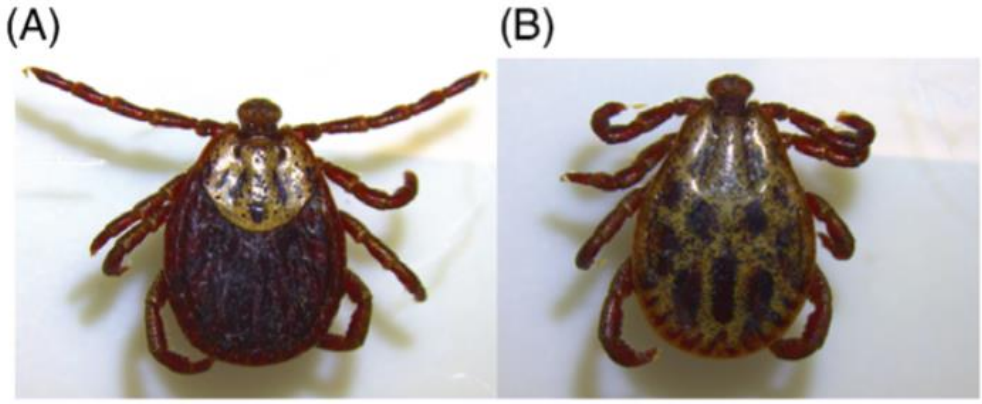


Fig 1.2 *Dermacentor reticulatus* (A) female (B) male (Medlock *et al.,* 2017).

Morphologically, *D. reticulatus* most resembles *D. marginatus,* the only other species of *Dermacentor* found in Europe, however the latter are generally smaller (Rubel *et al*., 2016). *Dermacentor* characteristically have shorter palps than *Ixodes*, ornate scuta, a palpal spur, and both males and females present white enamel ornamentation, with male ornamentation covering the entire scutum (Fig 1.2). Identifying immature instars is difficult as they resemble juveniles of other genera (Földvári *et al.,* 2016). There are 36 recognised species within the genus *Dermacentor* (Wang *et al.,* 2019).

Compared to more common UK ticks, such as *I. ricinus*, the ecology of *D. reticulatus* is less well known; there is a particular paucity of data relating to the immature instars which are almost never collected in blanket-drags. *Dermacentor reticulatus* has a three-host life cycle which takes approximately 1-2 years to complete (Rubel *et al*., 2016) and it has been suggested that its

development from larva to nymph is more rapid than other tick species such as *I. ricinus* (Randoph *et al.,* 1999). *Dermacentor* females usually oviposit between 3,000 and 7,500 eggs, depending on the species, and the degree of engorgement. As with other tick species, the eggs are covered with secretions from the Gené's organ to prevent desiccation (Sonenshine and Mather, 1994; Šimo *et al.,* 2004). High levels of mortality in the environment, thought to surpass 99.9% within the larval and nymphal instars, limits increases in abundance and distribution (Sonenshine and Mather, 1994). In contrast to *I. ricinus,* the larvae and nymphs are thought to exhibit nidicolous behaviour, remaining in the burrows or nests of their hosts (Pfäffle *et al.,* 2015). Although not currently proven, it is thought that larvae may remain deep in leaf litter or within burrows. This theory is supported by the absence of larvae from blanket drags. When they are recovered, the immature stages of *D. reticulatus* are found on small mammal hosts, often in mid-summer, with oviposition presumed to have occurred in spring (Pfäffle *et al*., 2015). Egg hatch occurs 12-19 days after oviposition (Nosek, 1972). Larvae feed for 2.5-6 days and nymphs generally for a longer period, around 4-12 days. The activity of the adults generally begins in late August/September through to April/May, only disrupted by changes in climatic variables such as falls in temperature (Rubel *et al*., 2016). Throughout the winter, *D. reticulatus* undergo hormonally controlled diapause to halt development during the unfavourable colder conditions (Gray *et al.,* 2016). It has been suggested that *D. reticulatus* begins activity after diapause earlier in spring than other species because they are able to withstand cooler temperatures (Tokhov *et al*., 2014). Unusually for a three-host tick species, overwintering has been observed for *D. reticulatus* on a host (Karbowiak *et al.,* 2003).

*Dermacentor reticulatus* quests and will attach to a wide range of hosts (Sonenshine and Mather, 1994). It is thought that different species act as the primary host depending on the type of habitat and geographical location of the tick population. The most common hosts in the UK include dogs, horses, cattle and, rarely, humans (Medlock *et al.,* 2017). The average questing height is 55cm (Menn, 2006) and possible hosts are detected using odour cues. For this reason, *D. reticulatus* are often found alongside paths used by domestic dogs and wildlife (Tharme, 1993). Male ticks remain on a host for a longer period than females, around 2-3 months, in order to mate with females, thus female ticks are more frequently observed questing than males (Tharme, 1993; Kiszewski *et al.,* 2001). When feeding, adult *D. reticulatus* ticks aggregate causing visible wounds with local inflammation (Buczek *et al*., 2015). This aggregation is deemed to be a consequence of

the production of aggregation-attachment pheromones, as demonstrated in other ticks (Petney and Bull, 1981) but not yet investigated in *D. reticulatus.*

### 1.5.2 Distribution

*Dermacentor reticulatus* is thought to be among the top three most abundant tick species in central Europe, along with *D. marginatus* and *I. ricinus* (Rubel *et al.,* 2014; Rubel *et al.,* 2016). However, it is relatively rare in the UK; a recent national survey of ticks on dogs in the UK detected only three *D. reticulatus* infestations from a sample of over 8,000 (Abdullah et al., 2016). It is thought that the distribution of *D. reticulatus* in Europe (Fig 1.3) is divided into two genetically distinct groups, with a Western European population and an Eastern European population, with an overlap of the two groups in Poland (Paulauskas *et al.,* 2018). This tick is found in much of continental Europe including, but not exclusively in, Hungary (Földvári and Farkas, 2005; Sréter *et al.,* 2005), Czech Republic (Nosek, 1972; Hubálek *et al.,* 2003), Germany (Menn, 2006; Rubel *et al.,* 2014; Pfäffle *et al.,* 2015), Slovakia (Nosek, 1972; Bullová *et al.,* 2009; Špitalská *et al.,* 2012), Poland (Bartosik *et al.,* 2011; Wójcik-Fatla *et al.,* 2011; Biernat *et al.,* 2014; Mierzejewska *et al.,* 2015b; Mierzejewska *et al.,* 2016), Switzerland (Perret *et al.,* 2000), Italy (Olivieri *et al.,* 2017), Russia (Tokhov *et al.,* 2014) and France (Martinod and Gilot, 1991).

It has been suggested that the distribution of *D. reticulatus* is expanding north through Europe (Rubel *et al.,* 2016). Newly established populations have been observed in Poland (Zygner *et al.,* 2009), Germany (Dautel *et al.,* 2006) and The Netherlands (Matjila *et al.,* 2005).

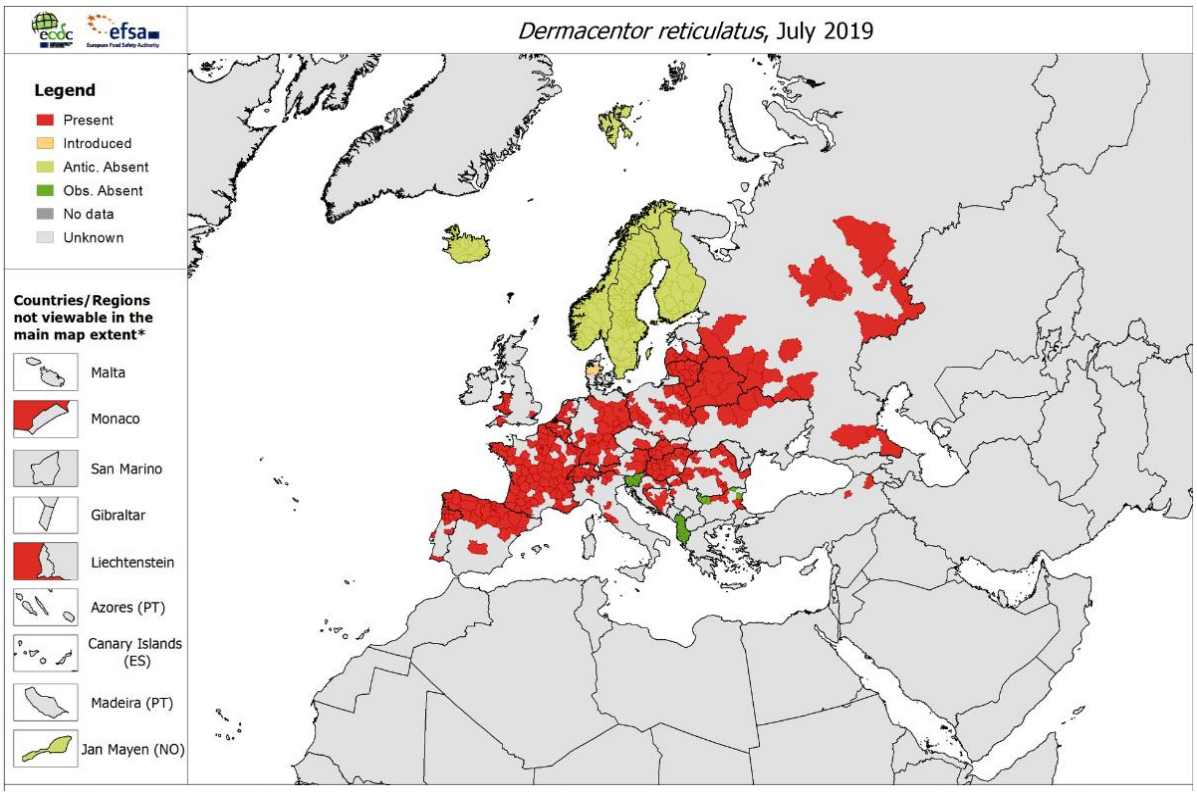


Fig 1.3 Current *D. reticulatus* geographical distribution in Europe detailing where *D. reticulatus* is present, has been introduced, and is absent based on data provided by the Vector-Net project and published historical data (from ECDC and EFSA, 2019)

In Europe, it has been suggested that *D. reticulatus* is largely associated with alluvial forest and swamp habitats, and the species is able to survive submergence for extended periods of time (Rubel *et al.,* 2016). Eggs have even been observed to survive in basins of rainwater (Hoogstraal, 1967). However, established populations may also survive in dry conditions, such as on heathland and grassland, and are rarely observed in dark or coniferous forests, unlike *I. ricinus* which is more tolerant of closed, forested environments (Cerny *et al*., 1982; Rubel *et al*., 2016).

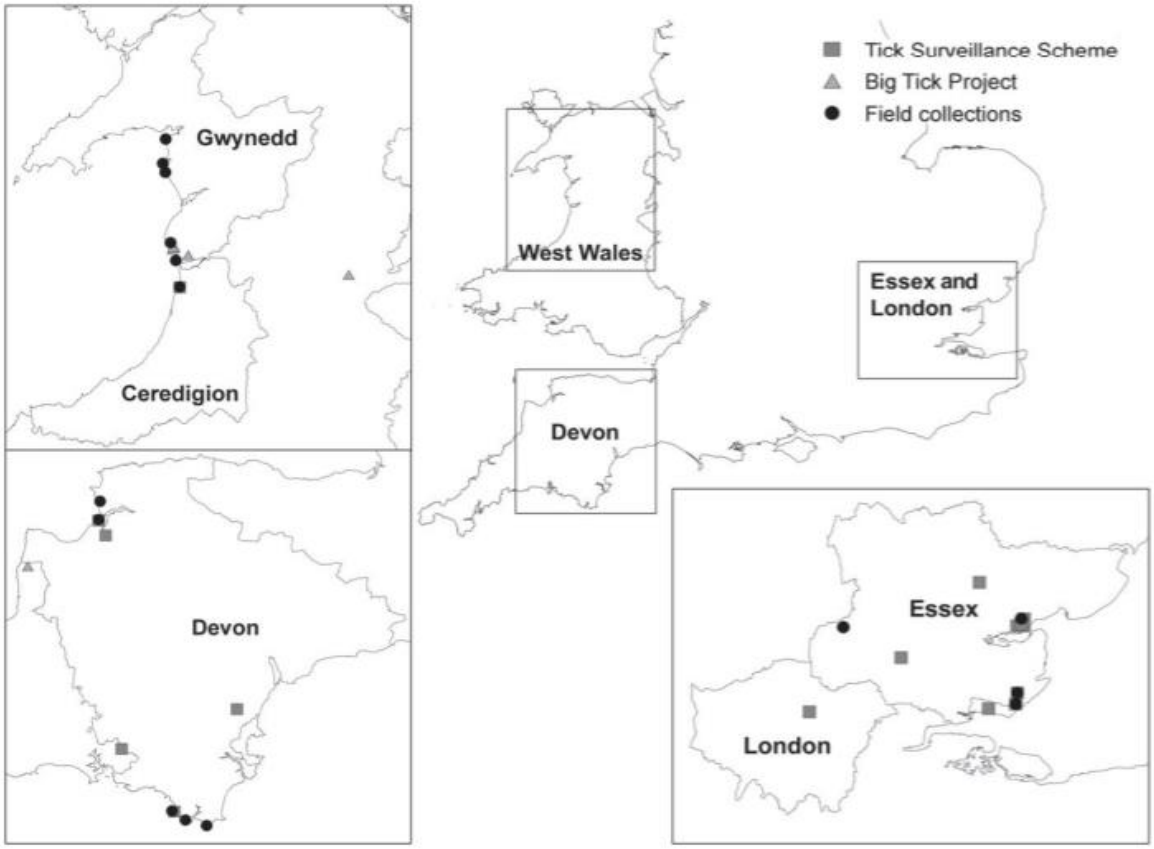


Fig 1.4 Current known distribution of *Dermacentor reticulatus* in the United Kingdom using data from Public Health England, the Big Tick Project and field collections between 2009-2016. The symbols show where *Dermacentor reticulatus* populations were reported to be by the mentioned sources (Medlock *et al.,* 2017).

The north-western border of the current distribution of *D. reticulatus* is the UK. It is thought that *D. reticulatus* has been present in small populations in the UK for over 100 years according to the Health Protection Agency (HPA) and British Records Centre (BRC) recording scheme (Medlock *et al.,* 2011), although the species may have been present for longer than this. According to historical records, questing *D. reticulatus* have been recorded in every month of the year in the UK, primarily during September to June with an activity peak in March and April (Medlock *et al.,* 2017).

In the UK, *D. reticulatus* is thought to be associated primarily with maritime grassland and sand dune habitats, including grazing marsh, and has been recorded in coastal sites in Devon and Wales (Medlock *et al.,* 2017). Historical and current records also suggest that many of the sites in the UK where this species is found are old rabbit warrens (Medlock *et al.,* 2017). Recently, *D.*

*reticulatus* has become more common in Wales and southern England (Fig 1.4). There are now four confirmed UK populations; the coast of West Wales between Harlech and Borth where their habitat is dominated by marram grass (*Ammophilia arenaria*) and Burnet Rose (*Rosa pimpinellifolia*), within parks and coastal grassland in Essex around Potton Island and Harlow, and on both the North and South Devon coasts namely in the Bideford sand dunes and Bolt Tail grassland (Medlock *et al.,* 2017; Medlock *et al.,* 2018). A population near Harlow in Essex, found recently in an area of grassland, is thought to be relatively newly established; it has been suggested that this may be linked to the movement of livestock and dogs. It has been suggested that *D. reticulatus* may continue to spread in this area (Medlock *et al.,* 2017). The establishment of this population suggests that the presumed association between *D. reticulatus* and coastal dune grassland in the UK may be incorrect. Alternatively it may be that the Harlow population is only transitory and will die out.

### 1.5.3 Epidemiological Impact

*Dermacentor reticulatus* is of increasing interest due to its rising epidemiological significance worldwide. It is suggested that there are over 40 different pathogens, known to infect both animals and humans, that have been detected in *D. reticulatus*, globally (Földvári *et al*., 2016). This tick species is thought to be an effective vector due to its high reproduction rate, rapid development rate, promiscuous feeding, and ability to survive in adverse conditions (Földvári *et al.,* 2016).

Pathogens transmitted by *D. reticulatus* in Eurasia include tick-borne encephalitis virus, *Rickettsia spp.*, *Anaplasma spp.* (Zivkovic *et al.,* 2007), *Babesia spp.*, *Theileria equi*, Kemerovo virus, bluetongue virus, *Borrelia spp.*, and *Francisella spp.* (Földvári *et al.,* 2016; Rubel *et al*., 2016). When considering the future threats from of veterinary disease that could become problematic in the UK, the most significant pathogens carried by *D. reticulatus* are *Babesia canis, Theilaria equi* and *Babesia caballi.* Additionally, *Rickettsia raoultii* has recently been detected in *D. reticulatus* populations in the UK (Tijsse-Klasen *et al.,* 2011*,* 2013).

### 1.5.4 Risks to veterinary and public health

*Canine babesiosis*

The apicomplexan piroplasmid parasite *Babesia canis* is the most notable pathogen spread by *D. reticulatus* and the causal agent of canine babesiosis, also known as piroplasmosis. Infection in dogs' results in a range of clinical signs, including fever, jaundice, anaemia, inflammation and, in severe cases, organ failure and death (Irwin, 2009). *Babesia canis* is capable of transovarial and transstadial transmission within ticks (Gray *et al.,* 2019), allowing *D. reticulatus* populations to act as effective reservoir hosts, maintaining this pathogen in the environment. The evidence suggests that wildlife cannot act as reservoirs, however domestic dogs can. Thus, it is thought that dogs are capable of transmitting the pathogen to feeding ticks and are, at least partially, responsible for its continuing dispersion. Vertical transmission of *B. canis* from female dogs to their litter has recently been observed, which intensifies the spread (Mierzejewska *et al.,* 2014). *Babesia canis* was detected for the first time in the UK in 2016, in a population discovered in Harlow, Essex (Swainsbury *et al.,* 2016). Subsequently, the prevalence of infected *D. reticulatus* ticks at this site was shown to be over 85% (de Marco *et al.,* 2017). It is likely that an infected dog introduced *B. canis* in this area and that the population of ticks at Harlow have sustained the pathogen via transstadial and transovarial transmission (Mierzejewska *et al.,* 2014).

*Babesia canis* was only isolated from a single *D. reticulatus* tick during a study in which 4750 ticks sent in by veterinary practices across the UK were examined for pathogens (Abdullah *et al.,* 2018). However, this tick came from a dog which had recently travelled back from France, emphasising the threat posed by untreated travelled dogs importing pathogens from other countries, including *B. canis*. This presents a serious veterinary health problem in the UK (Medlock *et al.,* 2018).

*Equine piroplasmosis*

*Dermacentor reticulatus* is a known vector of equine piroplasmosis caused by *Theileria equi* and *Babesia caballi*. These cause disease in equids, especially horses. Both pathogens can cause anaemia, neonatal death, and a high risk of mortality among other clinical signs (Guidi *et al.,* 2015). Recovered horses can act as reservoirs, allowing the further transmission to ticks. Sexual

development of *T. equi* and *B. caballi* occurs within the tick mid-gut and in its salivary glands. Both transstadial and transovarial transmission occurs, resulting in persistence within tick populations. Therefore, the distribution and spread of these pathogens is closely associated with the distribution and spread of their tick vectors (Scoles and Ueti, 2015). Despite protocols, the movement of infected horses across borders contributes considerably to the continuing spread of these pathogens, exposing them to naïve tick populations in new regions (Guidi *et al.,* 2015). Historically, equine piroplasmosis has not been detected in the UK and only small populations of suitable vectors have been present. Hence, *B. caballi* and *T. equi* have been of little concern. However, the recent increased number of recordings of *D. reticulatus* in the UK suggests that the potential for the status of equine piroplasmosis to change is high, should the pathogens be inadvertently introduced. A recent study concluded that horses imported to the UK are not monitored carefully enough; given that 8% of submitted equine samples tested positive for pathogen DNA, the proportion of infected animals was likely to have been considerably higher, particularly given the lack of sensitivity of the PCR test available (Coultous *et al.,* 2019).

*Rickettsia spp.*

Rickettsiae are intracellular bacteria with more than 20 confirmed species, 14 of which infect humans (Tijsse-Klasen *et al.,* 2011), causing a variety of syndromes such as tick-borne lymphadenopathy (TIBOLA) (Silva-Pinto *et al.,* 2014). The genus is separated into 3 distinct groups, one being the spotted fever group (SFG), the majority of which are transmitted by ixodid ticks, transmission occurring during feeding, but also transovarially and transstadially. Tick vectors of *Rickettsia* spp. have been identified on migratory birds in the UK, indicating that the long-distance transportation of ticks on migratory birds has the potential to introduce new pathogens into UK tick populations (Graham *et al.,* 2010). One study found that 27% of ticks tested were infected with *Rickettsia* spp., the majority being *Rickettsia raoultii,* in *D. reticulatus* collected from a variety of locations across the UK (Tijsse-Klasen *et al.,* 2011). Currently, SFG rickettsiae in *D. reticulatus* have been detected in Essex and Wales, but they are likely also to be present elsewhere in the UK (Tijsse-Klasen *et al.,* 2013). *Rickettsia slovaca* has been confirmed as present in the UK (Pietzsch *et al.,* 2015) and *D. reticulatus* has been identified as a possible vector (Špitalská *et al.,* 2012; Földvári *et al.,* 2013). The presence and establishment of these pathogens in the UK and the ability of *D.*

*reticulatus* to act as a vector and reservoir host suggests that *Rickettsia* spp. may pose serious public and veterinary health risks.

*Tick-borne encephalitis virus*

Tick-borne encephalitis virus (TBEV), an infection of the central nervous system, poses a public health threat in Europe and the majority of Asia. TBEV is classified within the genus of *Flavivirus*; it causes chronic fever, muscle pains, fits, exhaustion, paralytic disorders in its host and in rare cases infection can be fatal, depending on the form of TBEV and the age and genetics of the host (Gritsun *et al.,* 2003).

TBEV persists in ticks through transstadial, transovarial, viraemic and salivary assisted transmission (Gritsun *et al.,* 2003). Currently, the role of *D. reticulatus* in TBEV transmission is considered to be less important than that of *I. ricinus*. However, *D. reticulatus* has been implicated as a potential reservoir host of TBEV in Poland. Biernat *et al.* (2014) found a TBEV infection rate within *D. reticulatus* varying between 1 and 12% in all locations studied. An earlier study had found a 10.8% infection rate in *D. reticulatus*, which was higher than observed in *I. ricinus* (Wójcik-Fatla *et al.,* 2011). It is thought that *D. reticulatus* is more prevalent on cattle than *I. ricinus* and that cattle act a reservoir of TBEV, thus exacerbating the spread of TBEV by *D. reticulatus* (Mierzejewska *et al.,* 2015b). Recently TBEV has been detected in ticks in the UK for the first time in an area bordering Dorset and Hampshire (Public Health England, 2019). Though the current risk to public health is deemed low, the establishment of TBEV among cattle and *D. reticulatus* populations could make TBEV in the UK a considerable public health threat.

### 1.5.5 Environmental requirements

Nearly all species of tick are adapted to a specific climatic window, associated with particular habitats (Sonenshine and Mather, 1994). Optimal environmental conditions considerably improve the survival and development rate of ticks off-host (Needham and Teel, 1991). Humidity and temperature are particularly important, as these two variables together determine the rate of desiccation (Estrada-Peña, 2015). Together temperature and humidity can be expressed as an integrated measure, saturation deficit, representing the drying power of the atmosphere.

Saturation deficit is the amount by which the water vapor in the air must be increased to achieve saturation at any given temperature (Randolph and Storey, 1999). The amount of water air will hold at saturation increases with rising temperature and thus the same relative humidity will correspond to a greater saturation deficit at warm temperatures. Saturation deficit provides important information about the amount of water available in the atmosphere to ticks at any given temperature.

*Dermacentor reticulatus* has been observed to have a high tolerance to starvation; it has been suggested that adults can survive longer than 3 years without a host (Földvári *et al.,* 2016) and for 2.5 years post-moulting (Razumova, 1998). Thus, it is thought that tick abundance is controlled primarily by abiotic factors, secondarily by predation.

*Hosts*

Host availability determines the presence or absence and abundance of tick populations, as suitable hosts are required for successful tick development, feeding and reproduction (Estrada-Peña, 2015). A range of different species have been recorded as hosts for *D. reticulatus*, depending on climate and habitat type. There are four main locations in which *D. reticulatus* has been recorded in the UK, particularly along the coast of Wales (Medlock *et al.,* 2017). Past studies have found *D. reticulatus* populations close to camping grounds at Shell Island and in the vegetation alongside paths near golf courses where dogs often visit at Morfa Harlech, suggesting that dogs might be important hosts for questing adult ticks (Evans, 1951). This supports observations by Tharme (1993) that *D. reticulatus* is often found exhibiting host-seeking behaviour on the margins of habitat where there are high densities of suitable hosts, such as popular dog walking paths. It is thought that areas such as these have a higher density of ticks due to the tendency of host animals to repeatedly use particular paths or sites (Estrada-Peña, 2015). It is notable that grazing livestock are also present in all four known *D. reticulatus* locations in the UK, indicating that large mammals such as cattle and horses may also be hosts for adults (Medlock *et al.,* 2017).

Hosts for *D. reticulatus* larvae and nymphs in the UK are thought to include voles, moles, shrews, hares, hedgehogs, birds and rabbits while nymphs have also been recorded on weasels, polecats, cervids, goats and dogs (Nosek, 1972; Földvári *et al.,* 2016).

*Photoperiod*

Photoperiod is considered an important factor for inducing various behaviours in *D. reticulatus* (Tharme, 1993; Bartosik *et al.,* 2011). Mild winters may be insufficient to initiate winter diapause in Europe, which is thought to be triggered by declining temperature (Tharme, 1993). Additionally, photoperiod may stimulate the beginning of a summer diapause and then reactivate questing activity in August and September, as questing begins before the temperature decline, thus an increase in daylight hours is thought to be the primary stimulus (Tharme, 1993). Photoperiod both prevents and stimulates host-seeking activity of unfed *D. reticulatus*, as well as initiating a cessation of development in engorged ticks, enabling survival throughout adverse weather conditions (Bartosik *et al.,* 2011; Estrada-Peña *et al.,* 2013). There is also evidence that some ticks, such as *I. ricinus,* are active in the dark; one study found that only 1% of all movement occurred during maximum light intensity (Perret *et al.,* 2013). Therefore, it is possible that light intensity may have some similar influence over *D. reticulatus* activity, although this has not yet been investigated. Movement, primarily during darkness, could be a behaviour that limits tick desiccation (Perret *et al.,* 2013) while allowing questing activity to coincide with that of their hosts.

*Humidity*

Maintenance of body water is vital for terrestrial arthropods due to a large surface-to-volume ratio, making them particularly prone to desiccation. The relative abundance of host-seeking ticks is impacted by a multitude of factors, the most influential of which is thought to be relative humidity (and saturation deficit); changes in the numbers of questing ticks are strongly correlated with changes in saturation deficit (Randolph and Storey, 1999; Perret *et al.,* 2000; Randolph *et al.,* 2002; Hubálek *et al.,* 2003; Perret *et al.,* 2003). Ticks have a multitude of mechanisms guarding against water loss in desiccating environments, including the integument which acts as a highly efficient physical barrier against water loss (Meyer-König *et al.,* 2001b). Their respiratory system shielded from the environment via spiracular valves and the excretion of nitrogenous waste in the form of guanine crystals also limit water loss. Rehydration is achieved through vapour absorption, feeding and via metabolic activity (Needham and Teel, 1991). Tick saliva also has a function in water balance, containing a hygroscopic substance that absorbs water

vapour from the atmosphere (Nuttall, 2019). During host-seeking activities, water loss and energy consumption are increased thus limiting survival. It is imperative that ticks can survive until a suitable host is found (Meyer-König *et al.,* 2001a). Should the relative humidity fall so that ticks are unable to absorb water efficiently from the atmosphere, the resultant desiccation increases the mortality rate. In more humid conditions therefore, ticks are able to quest for longer as the desiccation rate is slower (Estrada-Peña et al., 2013). The highest water loss tolerance recorded for any tick species is for *Amblyomma americanum,* the lone star tick, which can lose as much as 70% water mass before loss of locomotor ability occurs (Edney, 1977). Most ticks are only able to move vertically, so when they experience high levels of water loss while questing they move back down to the vegetation mat to rehydrate; frequent movement between vegetation interspersed by bouts of questing have been recorded in low humidity conditions. This movement requires energy from the tick's fat reserves which is restored by blood-feeding. Immature stages usually quest at lower heights within vegetation because they are more susceptible to desiccation and this may be associated with their larger surface area to volume ratio, higher metabolic rate, and smaller reserves of fat (Mejlon and Jaenson, 1997; Randolph and Storey, 1999).

It has been suggested that D. *reticulatus* may be more sensitive to desiccation than the more common *I. ricinus* and that it therefore has a strong association with bodies of water in the environment (Nosek, 1972; Hubalek *et al.,* 2003). In contrast, some research has suggested that *D. reticulatus* may be more resistant to desiccation than *Ixodes* spp., surviving for significantly longer periods in dry air before dying (Lees, 1946). It is thought that *D. reticulatus* require relative humidities exceeding 80% to permit active uptake of water vapour from the atmosphere (MacLeod, 1935; Meyer-König *et al.,* 2001b). There is limited research on whether age or sex have any influence on desiccation ability, however there is some evidence suggesting that the permeability of the tick integument increases with age (Needham and Teel, 1991) and that females have a higher rate of survival in drier environments than males (Meyer-König *et al*., 2001b). For *I. ricinus,* it is thought that the relative humidity must exceed 70-80% for survival (MacLeod, 1935). Other literature estimates that ticks will stop losing water via evaporation when the atmospheric moisture level reaches 92% RH (Lees, 1946).

In studies in eastern Europe, *D. reticulatus* mortality was found to increase with higher saturation deficit (Meyer-König *et al.,* 2001a) and activity was highest when the relative humidity

was at its highest, between 55-65% (Buczek *et al.,* 2017). In contrast, humidity was reported not to have a significant impact on the activity of *D. reticulatus* in eastern Poland, where temperature and photoperiod were the only variables correlated with activity levels (Bartosik *et al.,* 2011). Hence, clearer information is required on the relationship between relative humidity and survival of *D. reticulatus*. Most previous work on *D. reticulatus* has been undertaken in Eastern Europe, so it is important to also investigate this behaviour in the UK.

There is some debate on the impact of rainfall on tick activity and desiccation. Several studies based in Europe found no correlation between rainfall and *D. reticulatus* activity (Martinod and Gilot, 1991; Hubálek *et al.,* 2003), although both studies used weather data supplied by a local meteorological station instead of recording temperature of the vegetation where the ticks were found. In contrast, it has been suggested that a higher average rainfall inevitably results in a higher humidity, thus resulting in better conditions for *D. reticulatus* survival (Gerstengarbe *et al.,* 2008, Süss *et al.,* 2008). It has also been argued that rainfall increases the activity of *D. reticulatus* after diapause (Olivieri *et al.,* 2017).

*Temperature*

Temperature has been shown to have a significant effect on *D. reticulatus* activity and mortality in France and Poland (Martinod and Gilot, 1991; Bartosik *et al.,* 2011). However, a weakness of many studies is, as mentioned above, that they use data from local weather stations, which may not be a true representation of the temperature of the vegetation layer close to the ground where the ticks are found (Estrada-Peña *et al.,* 2013; Boehnke *et al.,* 2017). Vegetation temperature has been found to have a significant impact on the activity of *D. reticulatus* as it influences the saturation deficit, thus affecting activity and mortality rates (Tharme, 1993; Estrada-Peña, 2015).

In western Siberia, the most easterly region of the known range of *D. reticulatus*, adults have been observed host-seeking for only 3 months during spring and 3 months during the summer. Diapause, or quiescence, halts development during unfavourable conditions (Gray *et al.,* 2016). Thus, questing ticks are not found in these areas between September and April, due to the harsh winter temperatures. In the UK, the most westerly distribution of this species, *D. reticulatus* appears to have a short summer diapause between June and August, and this is thought to occur

due to unfavourably warm temperatures and low humidity. The brief winter period when ticks are not found questing is probably not a true diapause, as adult *D. reticulatus* can reappear very quickly when the weather becomes milder and they can sometimes continue questing throughout winter (Tharme, 1993). Occasionally during severe winters, long periods of heavy snow cover on top of the litter provides protection, insulating the ticks against low temperatures (Estrada-Peña, 2015).

*Dermacentor reticulatus* has been shown to be active during the winter months in temperatures that are too cold for *I. ricinus,* suggesting that *D. reticulatus* is cold-hardy compared to other ixodid tick species (Tharme, 1993). The threshold temperature for *I. ricinus* activity is thought to be 7°C (Tharme, 1993; Perret *et al.,* 2000). Active questing of *D. reticulatus* ticks has been observed at temperatures as low as 1°C and a soil temperature of -0.1$^{o}$ C in mainland and Eastern Europe (Nosek, 1972; Hubálek *et al.,* 2003). While monitoring questing at a site in Wales over a period of 24 hours, the lowest temperature recorded at which *D. reticulatus* was still questing at was 3.3°C and the overnight temperature was -5.4°C, and the surface of the sand at the marsh site was frozen (Tharme, 1993). However, this latter study only monitored this activity over a single night and previous studies have primarily used data from weather stations rather than the vegetation layer. While using weather station data is accurate to some extent, as atmospheric conditions affect the conditions in the vegetation and affect ticks during questing, it is thought that using data recorded at vegetation height is preferred, as this is where ticks rehydrate. If the climate with the vegetation is unsuitable, then the ticks cannot survive in that location. In this way, the temperature threshold for *D. reticulatus* questing activity remains unclear. The majority of studies on *D. reticulatus* are based in eastern and central Europe, mostly in Poland, Czech Republic and France, leaving the activity and mortality of this species under different temperature conditions undetermined in the UK.

### 1.5.6 Changing abundance and distribution

Over the past decade, many arthropod vectors, including *D. reticulatus*, are thought to have increased their abundance and expanded their distribution, despite on-going research and expenditure on ectoparasiticides. These changes are thought to be the result of the synchronous impact of many factors, creating more favourable environments for tick establishment (Karbowiak, 2014; Paulauskas *et al.,* 2018).

There are a multitude of elements that may influence the dispersion of *D. reticulatus* such as human migration, globalisation, land-use change, trade, and urbanisation (Karbowiak, 2014; Medlock *et al.,* 2015). Change in agricultural practices, such as a reduction in the use of pesticides, abandonment of agricultural land, and more reforestation can be favourable for *D. reticulatus* and other tick species (Karbowiak, 2014, Mierzejewska *et al.,* 2015a). There has also been a reported increase in tick abundance in urban greenspace especially, such as parks and small woodlands (Medlock *et al.,* 2018). These, along with other similar spaces contain a mosaic of vegetation and are an increasingly suitable habitat for the hosts of *D. reticulatus*. Thus, ticks that are dispersed by their hosts to new habitats are more easily able to establish a population (Karbowiak, 2014). Movement of animal hosts between sites using wildlife corridors such as river valleys, more regular travel to the countryside with domestic dogs, and an increase in livestock trading routes exacerbates the issue (Mierzejewska *et al.,* 2016; Rubel *et al.,* 2016; Medlock *et al.,* 2018).

Climate change is often highlighted as a key driver of changes in tick distribution globally and includes a multitude of variables such as changing patterns of temperature, wind direction and precipitation. Throughout Europe mean temperatures have increased, more rainfall has been recorded and seasons of optimum growth have lengthened resulting in a warmer, wetter northern Europe (Karbowiak, 2014). Thus, conditions are more favourable for tick species that were previously incapable of surviving in a cooler, more temperate environment. For instance, *Hyalomma marginatum,* the primary vector of Crimean-Congo haemorrhagic fever virus in Europe, enters the UK on migratory birds every year, however the climate has previously been too cold for it to survive. It has been suggested that in the near future, the UK could become warm enough for this tick to become established (Gale *et al.,* 2012).

## 1.6 Study aims

The aim of the research described in this thesis was to determine the effect of temperature and humidity on the survival of the tick *D. reticulatus* collected from UK populations. Ticks were to be collected from various sites on the west coast of Wales, north and south coasts of Devon, and Essex. As highlighted in the review above, the environmental constraints that determine the distribution and abundance of this species are poorly understood and very few studies have investigated *D. reticulatus* in the UK. Since this tick is an important vector of the causal agents of a

variety of diseases of considerable veterinary and public health concern, identifying its basic microclimatic requirements is vital. The data provided by this research will aid in analysis of the risk from *D. reticulatus* in the UK and provide information on the potential spread of emerging tick-borne pathogens. This knowledge will be useful for future population modelling vital for sustainable management of this vector.

# CHAPTER 2

## Effects of temperature and humidity on mortality

### 2.1 Introduction

As discussed in Chapter 1, the abundance and distribution of *D. reticulatus* is increasing, both in central Europe and in the UK (Matjila *et al.,* 2005; Dautel *et al.,* 2006; Zygner *et al.,* 2009; Rubel *et al.,* 2016; Medlock *et al.,* 2017). Climate change is thought to be a key driver in this changing pattern (Karbowiak, 2014). In the future, *D. reticulatus* may become more widely dispersed and abundant in the UK (Gray *et al.,* 2009; Jameson and Medlock, 2011) and the threat the pathogens it transmits may become more substantial (Földvári *et al.,* 2016; Rubel *et al*., 2016). Humidity and temperature are thought to be the most influential environmental factors affecting survival and abundance because together they determine the rate of desiccation (Randolph and Storey, 1999; Bartosik *et al.,* 2011). However, there is a lack of data or consensus on the effects of these factors on *D. reticulatus*. Therefore, the aims of this study were to examine the mortality rate of *D. reticulatus* collected from four locations under varying temperatures and humidities to better understand the basic climatic requirements and the effect that variations in these might have on the mortality of *D. reticulatus* in the UK as a whole.

### 2.2 Methods

#### 2.2.1 Collection sites

There are four known areas where D. *reticulatus* can be found in the UK; West Wales, Essex, North Devon, and South Devon (Medlock *et al.,* 2017). Ticks for this study were collected from sites within all four areas between September 2019 and February 2020.

Tick collection in Wales occurred along a stretch of coastline near Harlech. Harlech is located on the west coast of Wales within Snowdonia National Park. Tick collection took place in three areas. Behind Harlech beach there is a dune area at 52.8731° N, 4.1294° W containing some small bodies of water, grazing cattle and dog-walking paths. The dunes nearest the beach are

mostly devoid of plant life other than marram grass (*Ammophila arenaria)*. The majority of ticks were found in the dune slacks further from the beach where there are a variety of plant species and small bodies of water on flatter ground. The second collection site was around 5km south of Harlech on Shell Island at 52.8080° N, 4.1443° W, a peninsula on which there is a $1.2^2$ km campsite during the summer months and during the winter months local farmers graze sheep. Access to the island is only possible along a causeway across the River Arto estuary. Shell Island attracts many dog-walkers in the summer months. The majority of ticks were collected south of the main campsite in vegetative areas surrounding large sand dunes. The third collection site was in the dune area near Dyffryn Ardudwy at 52.7813° N, 4.1182° W, located in the dunes next to Benar Beach. Tick collection occurred primarily in sheltered, highly vegetative areas positioned behind the large sand dunes. Dog-walking trails are numerous here and cattle are grazed in pastures just behind the dune area in which the ticks were collected.

Braunton Burrows and Northam Burrows are on the north coast of Devon, west of Barnstaple. Ticks were collected from Braunton Burrows, a dune area of approximately 1400 hectares at 51.0928° N, 4.207895° W behind Saunton Sands beach. Across the river mouth to the south is Northam Burrows Country Park at 51.0545° N, 4.2245° W. Ticks were collected across both locations, in scrubland beyond the beach. Both areas are popular with dog-walkers and farm animals are periodically grazed there, usually cows in Braunton Burrows and horses in Northam Burrows.

Hope Cove is located on the south coast of the UK in Devon. Tick collection took place on the cliffs next to Hope Cove near Bolt Tail at 50.2427° N, 3.8660° W. There are numerous walking trails popular with dog-walkers. The ground on top of the cliffs is primarily covered by short grass; thicker, taller vegetation is located near the edges of cliffs looking over Hope Cove beach. This is where the majority of ticks were found.

Old Hall Marshes, an RSPB nature reserve at 51.7763° N, 0.8542° W, was the site in Essex where ticks were collected. This area contains many bodies of water, extensive saltmarsh, reedbeds and vast grazing marshes. The reserve is open to the public and is popular with dog-walkers.

The locations ticks were collected from differ in climate. The mean daily minimum temperature in January varies between sites. On Welsh coasts this temperature ranges between

3°-4°, whereas in eastern England it is 1°, and in Devon it's thought to vary between 1° -5° (Met Office UK, 2020). In contrast, in July, the mean daily maximum temperature is 18° on the Welsh coast, between 20-23° in Essex and upwards of 19° in the South-West. Along with temperature, rainfall also varies between the locations, from 1000mm annually along the Welsh coast, 900-1000mm on the south-west coast to less than 700mm annually in the east of England, sometimes as little as 500mm in the driest areas (Met Office UK, 2020).

### 2.2.2 Collection method

All ticks were collected using the standard blanket-dragging technique. A bamboo pole of approximately 1.2m had a 1m² white cotton blanket attached. On each end of the bamboo pole green twine was tied and used to pull the apparatus along the ground and over vegetation. The twine was approximately 2.5m long. The blanket acted as a makeshift 'host', the movement and texture imitating that of a roaming mammal. The blanket was dragged along the ground slowly and turned over to check for tick attachment. The usual protocol while collecting ticks such as *Ixodes ricinus* is to check for ticks every 10m, however *D. reticulatus* is a larger species and is easily knocked from the blanket. To limit this occurrence, the blanket was turned over every 5m. *D. reticulatus* were identified using key morphological features, primarily the white enamel ornamentation and the size of the tick. Forceps were used to remove the tick from the blanket and place it into collection tubes (Sarstedt, 30ml). Into each collection tube a blade of grass was placed for ticks to attach to during transport. There were normally between 15 and 30 ticks stored in each tube (Fig 2.1). If the collecting trip spanned 2 days, those collected on the first day were kept in a portable 4°C mini-fridge over-night. Once returned to the laboratory, the ticks were kept in their original collection tubes in a 4°C fridge until the experiment was set up. Each tick was used only once, and ticks remained in the fridge at 4°C for no longer than 3 days.

All *D. reticulatus* ticks collected were adults. As discussed in Chapter 1, larvae and nymphs are difficult to find in their natural habitats, as it is believed that immature stages remain in host burrows or on small mammal hosts. Other tick species collected on the blankets were not used in the study.

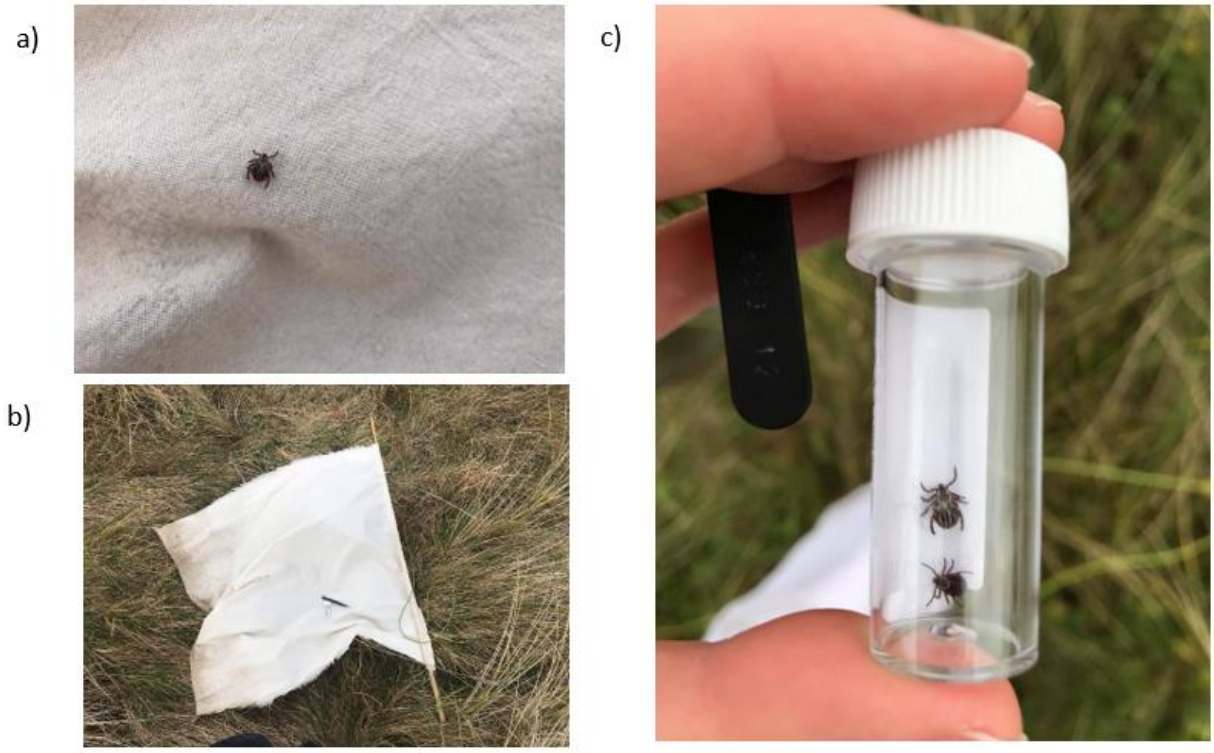


Fig 2.1 Collection of ticks: a) a male *D. reticulatus* on the blanket, having attached while the blanket was being dragged over vegetation; b) the blanket used for tick collection, made up of a 1.2m bamboo pole and 1m$^2$ cotton sheet; c) *D. reticulatus,* a male and a female, within a collection tube ready for transportation to the laboratory

### 2.2.3 Experiment design

During the study, glass desiccator jars (2.4L, VWR International) were used. Within each jar 3 tubes (50ml, Sarstedt) were placed and within each tube were 5 ticks of mixed sex and several 'grass stems' made out of filter paper (Whatman No. 1) for tick attachment. The lids of the tubes were removed and the top covered with fine mesh, secured with a rubber band allowing airflow between the jar and the ticks in the tube (Fig 2.2). Five different relative humidities (RH), 20% RH, 40% RH, 60% RH, 80% RH and 95% RH, were created by altering the concentration of potassium hydroxide KOH added to 100ml of water (Table 2.1) (Solomon, 1951). The solution was poured into the bottom of the jar and the tubes were placed on a mesh covering, preventing the tubes of ticks

falling into the KOH. Jars were placed in incubators set at 4°C (Leibherr, Medline), 15°C and 30°C (MLR-351H; Sanyo, Panasonic, Loughborough, UK).

The lid rim was covered by a fine layer of Vaseline to create a seal and once the KOH had been poured in and the ticks placed inside, the lid was quickly placed on the jar (Fig 2.2). The jar was then put in the incubator and remained there for 9 weeks (63 days) or until all the ticks inside were dead. Tick mortality was checked every 2 or 3 days and recorded. When the ticks were checked for mortality, the lid was replaced as quickly as possible after tubes were removed or put back in to ensure as little change in the internal humidity of the jar as possible. An EasyLog USB Data Logger (Lascar Electronics) was used to check that the humidity was correct in each jar and the KOH solution was changed every 2 weeks to ensure the humidity stayed at the desired level. The incubators used were kept dark inside during the investigation for consistency and so that light did not impact tick activity levels. The experiments were completed in stages between 4th December and 8th April (2019/2020), each one starting immediately after returning with new ticks from a field trip and continuing for the 9-week period. Overall, 225 *D. reticulatus* ticks were used in the study.

Table 2.1 The grams of potassium hydroxide that was added to 100ml of water, and the relative humidity created inside the jar where the ticks were kept after the lid was sealed, detected using an EasyLog USB Data Logger.

| KOH (grams) | Humidity RH% |
|---|---|
| 100 | 20 |
| 80 | 40 |
| 40 | 60 |
| 15 | 80 |
| 6 | 95 |

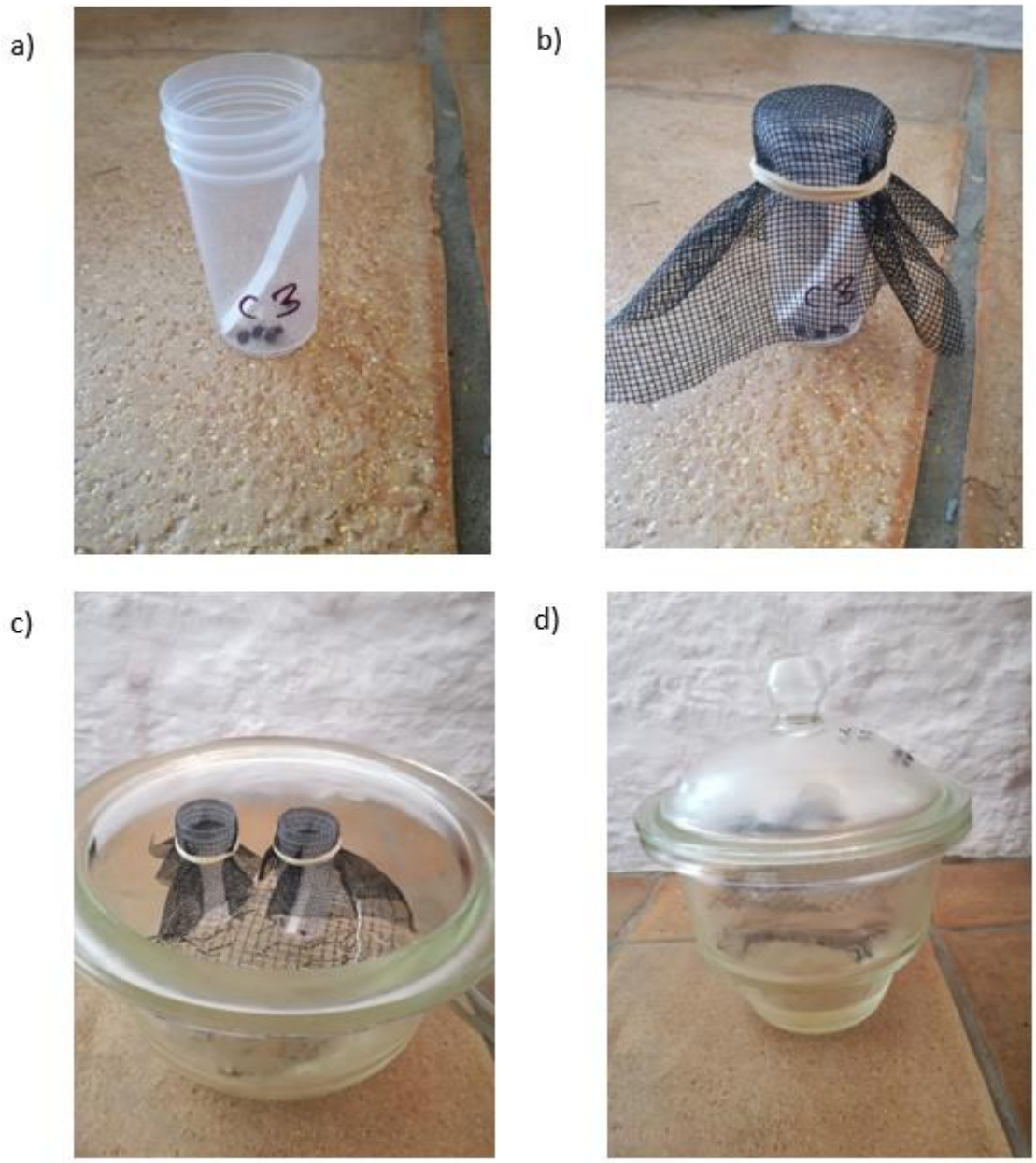


Fig 2.2 Desiccator jar assemblage: a) 5 ticks and a filter paper 'grass stem' within a tube; b) fine mesh to allow air flow was secured on top of a tube with a rubber-band to hold it in place; c) two tubes within a desiccator jar standing on top of mesh to prevent them falling into the solution below; d) a desiccator jar containing tubes of ticks with the lid sealed on top using Vaseline to keep the internal humidity from changing

### 2.2.4 Statistical analysis

During statistical analysis, the primary dependent variable was the number of dead ticks recorded. Poisson regression and a generalized linear model (GLM) were used to investigate how tick survival varied with humidity, temperature, and time. In the GLM temperature and humidity were inputted as continuous variables and count was the dependent variable; time was entered as a random factor to control for repeated measures. Data were analysed using RStudio (R, version 3.4.2, R Core Team 2017). Temperature and humidity were used to calculate the saturation deficit (Randolph and Storey, 1999) and the relationship between saturation deficit and count was investigated by Poisson regression. The Poisson distribution was found to be the best fit for dead tick count, and the model fulfilled the suitability requirements (residual deviance less than twice the residual degrees of freedom, no over or under-dispersion and normal distribution of standardised residuals). The ticks were exposed to the differing climatic variables for 63 days, so 5 time points were analysed using R Studio: day 10, day 20, day 30, day 50 and day 63.

## 2.3 Results

There was a significant interaction between humidity, temperature, and time on tick survival overall ($Z_{214}$=3.8, $P$<0.001) so each time point was then separately analysed. At day 10 (Fig. 2.3), there was no significant relationship between survival and humidity at any temperature, indicating that the ticks could survive for this time period even when the humidity was low and the temperature was high. At day 20, there was a significant interactive effect between survival and humidity overall ($Z_{44}$=4.4, $P$<0.001). While there was no significant relationship between survival and humidity at 4°C and 15°C, there was a significant relationship at 30°C ($Z_{14}$=4.6, $P$<0.001). At day 30, there was again a significant interactive effect between survival and humidity overall ($Z_{44}$=4.7, P<0.001; Fig. 2.5) and again this was caused by the fact that there was no significant relationship between survival and humidity at 4°C, but there was a significant relationship between survival and humidity at 15°C ($Z_{14}$=3.7, $P$<0.001) and at 30°C ($Z_{14}$=4.2, $P$<0.001). The relationships between survival and humidity at 4°C, 15°C and 30°C were all significant at day 50 ($Z_{14}$=3.6, $P$<0.001; $Z_{14}$=3.8, $P$<0.001; $Z_{14}$=3.6, $P$<0.001, respectively; Fig. 2.6) and day 63 ($Z_{14}$=3.9, $P$<0.001; $Z_{14}$=3.6, $P$<0.001;

$Z_{14}$=3.1, *P*<0.05 respectively, Fig. 2.7). The average number of ticks alive was calculated using the data from each of the three repeats and plotted for temperature and humidity at each time point.

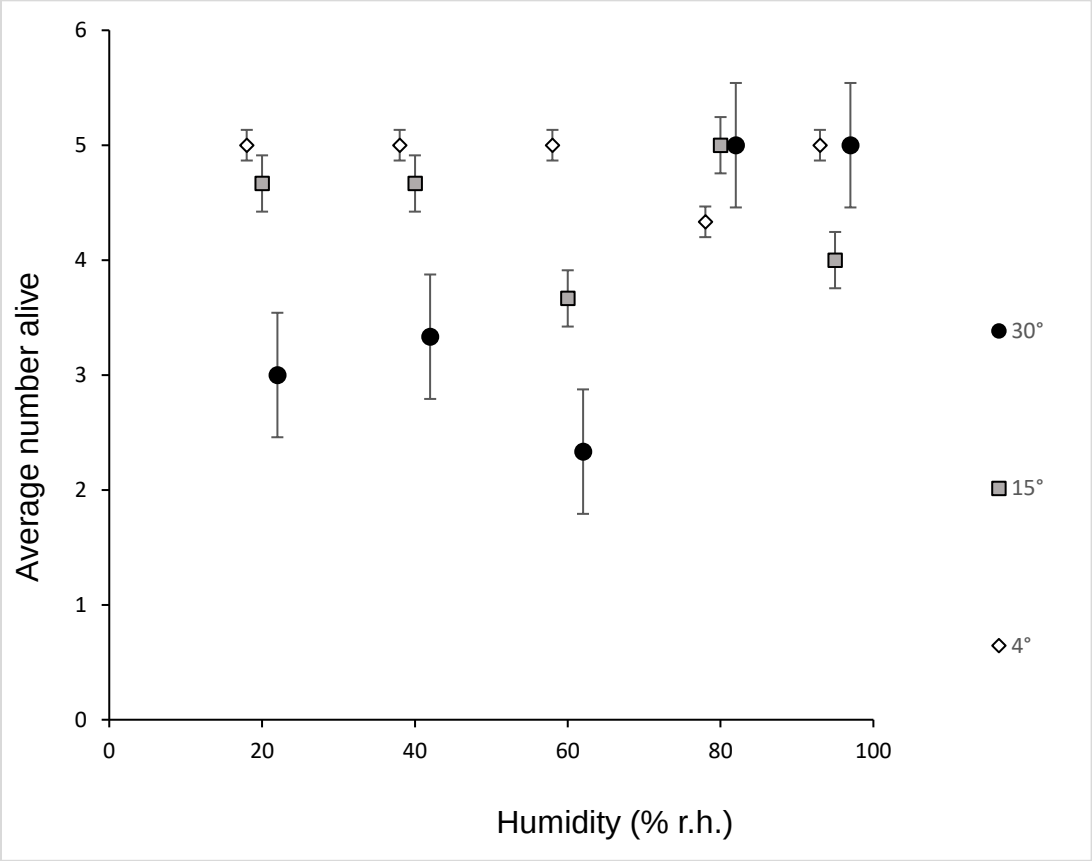


Fig. 2.3 The average number of ticks alive, calculated using the number of ticks alive from each of the three repeats, in different humidity (20, 40, 60, 80 and 95% RH) and temperature (4°, 15° and 30°) conditions on day 10. The error bars represent standard error of the mean.

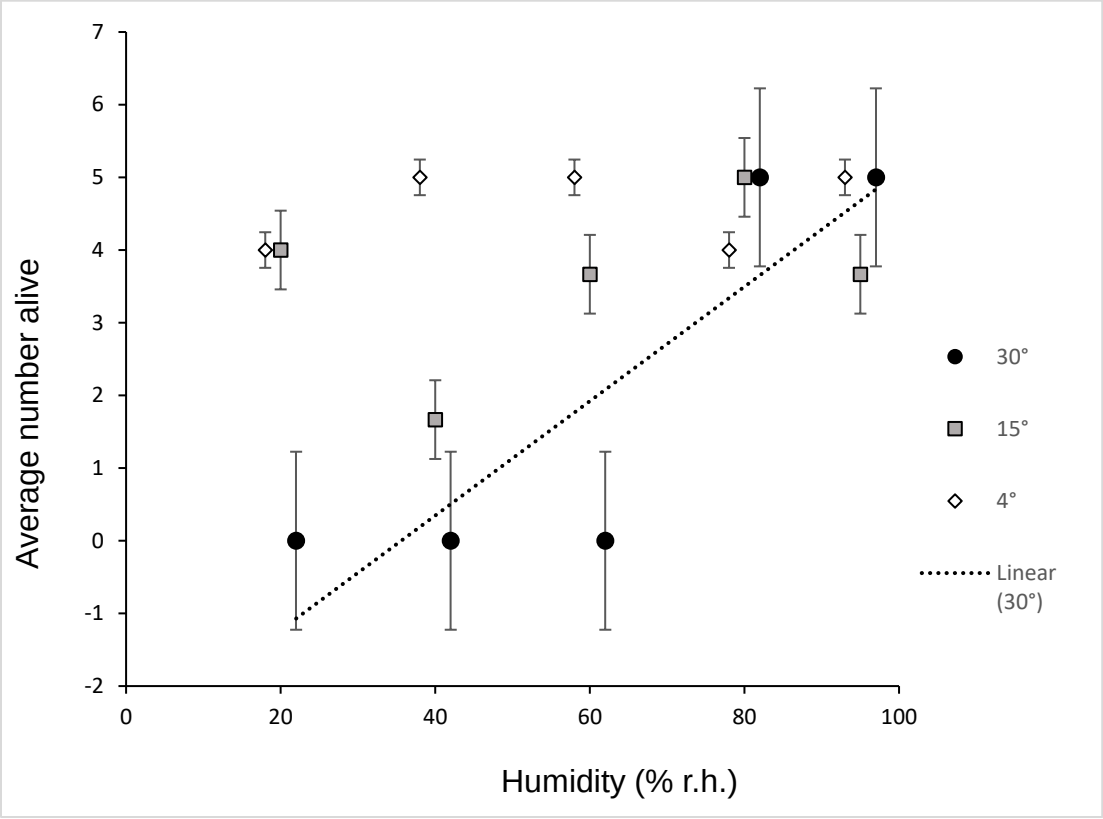


Fig. 2.4 The average number of ticks alive, calculated using the number of ticks alive from each of the three repeats, in different humidity (20, 40, 60, 80 and 95% RH) and temperature (4°, 15° and 30°) conditions on day 20 (30°C: Y=0.0787*X - 2.645, $Z_{14}$=4.6, *P*<0.001, $r^2$=0.7479). The error bars represent standard error of the mean.

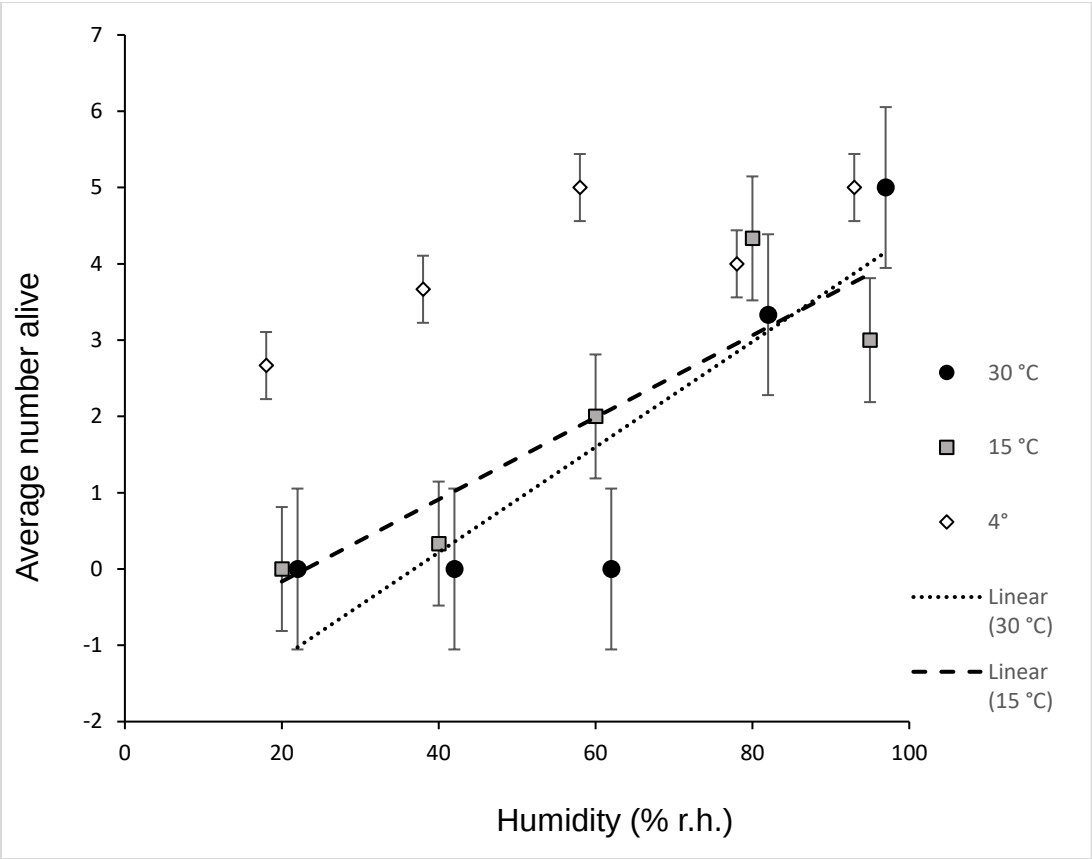


**Fig. 2.5** The average number of ticks alive, calculated using the number of ticks alive from each of the three repeats, in different humidity (20, 40, 60, 80 and 95% RH) and temperature (4°, 15° and 30°) conditions on day 30(30°C: Y=0.0619*X - 2.4079, $Z_{14}$=4.2, *P*<0.001, $r^2$=0.7261; 15°C: Y= 0.0538*X - 1.2394, $Z_{14}$=3.7, *P*<0.001, $r^2$= 0.6418). The error bars represent standard error of the mean.

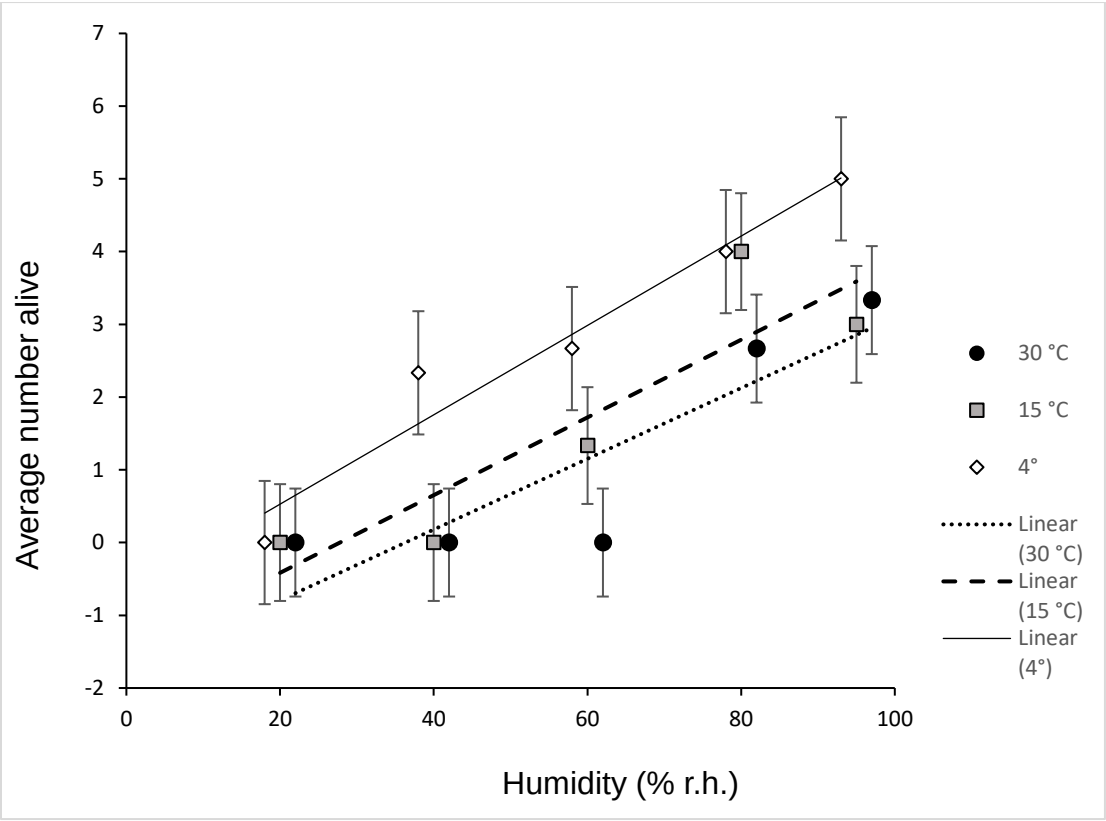


Fig. 2.6 The average number of ticks alive, calculated using the number of ticks alive from each of the three repeats, in different humidity (20, 40, 60, 80 and 95% RH) and temperature (4°, 15° and 30°) conditions on day 50 (30°C: Y=0.0486*X - 1.6685, $Z_{14}$=3.6, *P*<0.001, $r^2$=0.6685; 15°C: Y=0.0534*X - 1.4843, $Z_{14}$=3.8, *P*<0.001, $r^2$= 0.6544; 4°C:Y=0.0622*X - 0.7251, $Z_{14}$=3.6, *P*<0.001, $r^2$=0.6745). The error bars represent standard error of the mean.

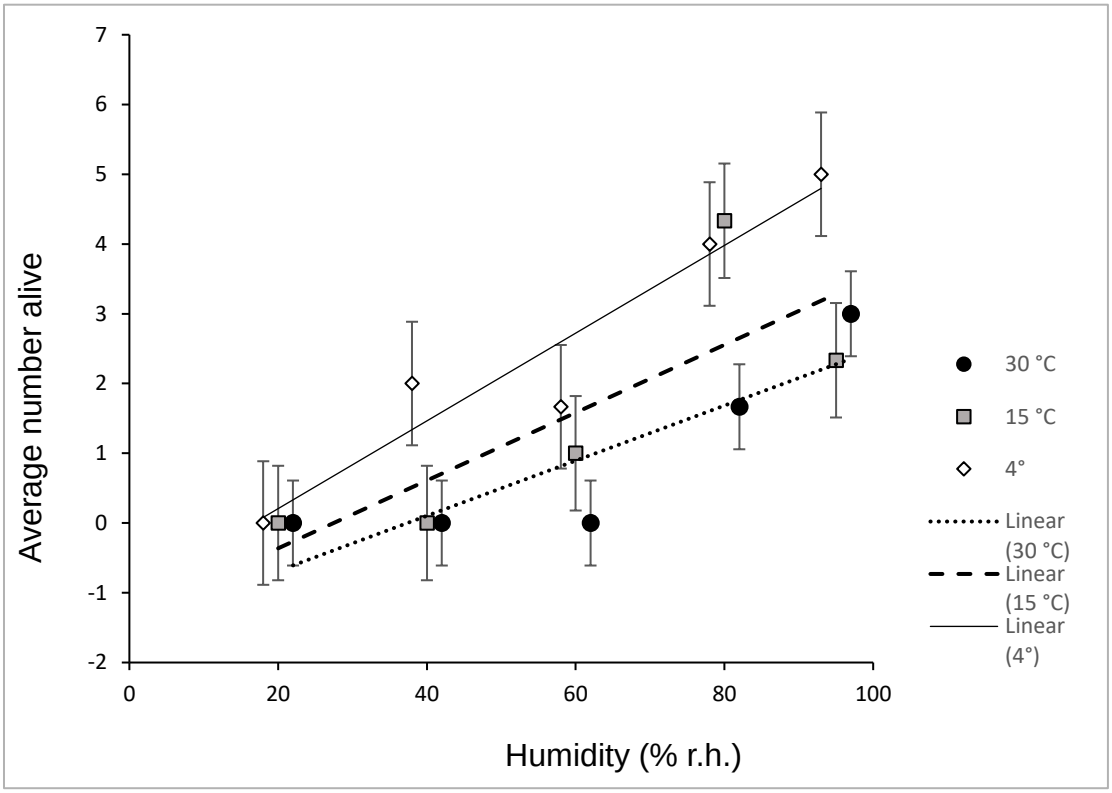


Fig. 2.7 The average number of ticks alive, calculated using the number of ticks alive from each of the three repeats, in different humidity (20, 40, 60, 80 and 95% RH) and temperature (4°, 15° and 30°) conditions on day 63 (30°C: Y=0.0395*X - 1.3973, $Z_{14}$=3.1, *P*<0.05, $r^2$=0.5478; 15°C: Y=0.0486*X - 1.3352, $Z_{14}$=3.6, *P*<0.001, $r^2$= 0.4606; 4°C:Y=0.0621*X - 1.1452, $Z_{14}$=3.9, *P*<0.001, $r^2$=0.6856). The error bars represent standard error of the mean.

The data were further explored by investigating the influence of saturation deficit on survival rate using a Poisson regression GLM for each time point. At day 10 there was no significant influence (Fig. 2.8). Saturation deficit was found to have a significant logarithmic relationship with survival rate for the remaining time points, at day 20 ($Z_{44}$=5.6, *P*<0.001; Fig. 2.9), day 30 ($Z_{44}$=6.1, *P*<0.001; Fig. 2.10), day 50 ($Z_{44}$=5.8, *P*<0.001; Fig. 2.11), and day 63 ($Z_{44}$=5.4, *P*<0.001; Fig. 2.12).

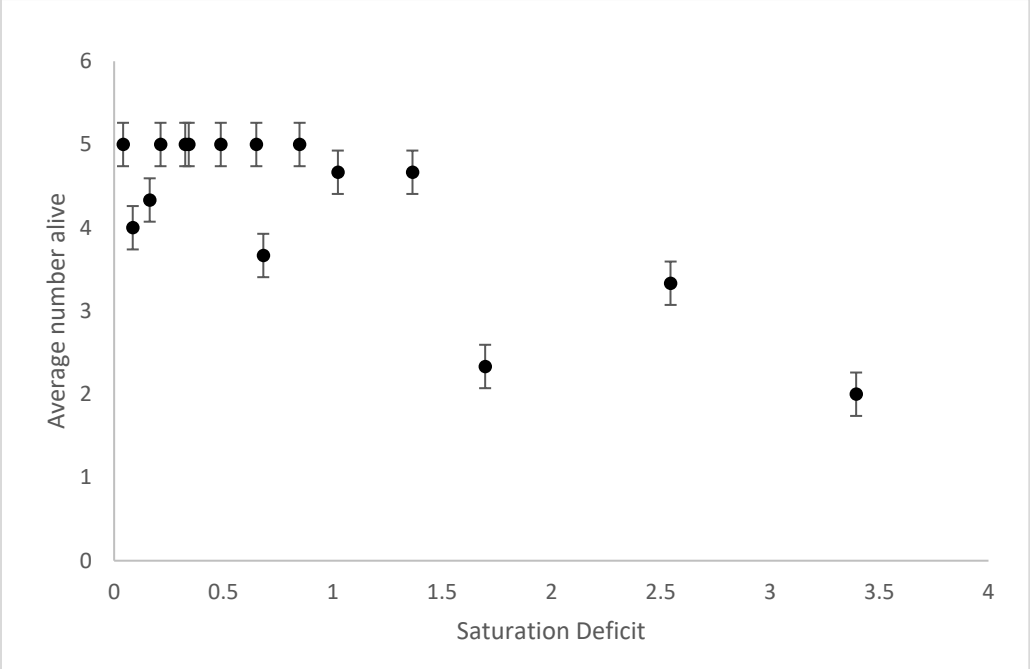


Fig. 2.8 The average number of ticks alive on day 10 and the corresponding saturation deficit of the jar in which the ticks were kept, the saturation deficit being an integration of the humidity and temperature and defined as the drying power of the air. The error bars represent standard error of the mean.

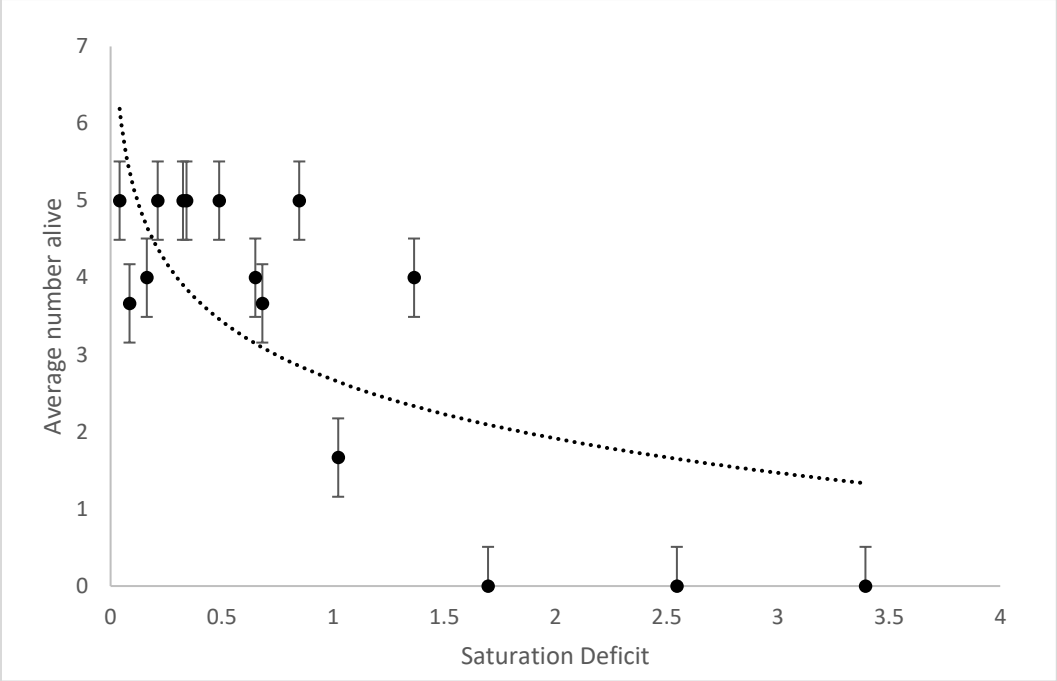


Fig. 2.9 The average number of ticks alive on day 20 and the corresponding saturation deficit of the jar in which the ticks were kept, the saturation deficit being an integration of the humidity and temperature and defined as the drying power of the air (Y=-1.098ln(X) + 2.6749, $Z_{44}$=5.6, *P*<0.001, $r^2$= 0.4079). The error bars represent standard error of the mean.

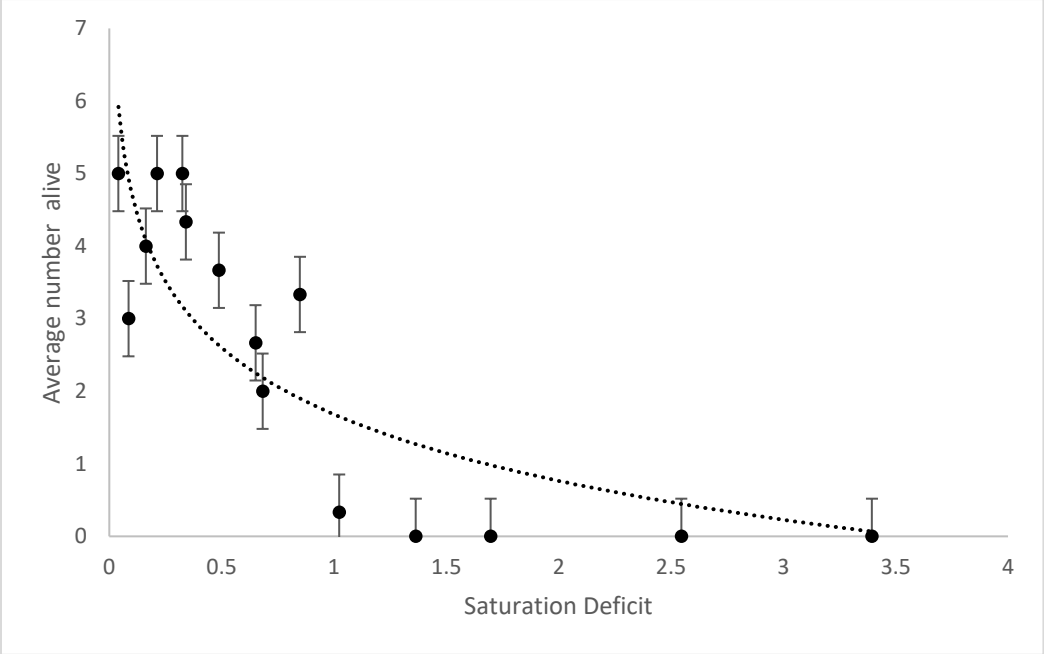


Fig. 2.10 The average number of ticks alive on day 30 and the corresponding saturation deficit of the jar in which the ticks were kept, the saturation deficit being an integration of the humidity and temperature and defined as the drying power of the air (Y=-1.323ln(X) + 1.682, $Z_{44}$=6.1, *P*<0.001, $r^2$= 0.5673). The error bars represent standard error of the mean.

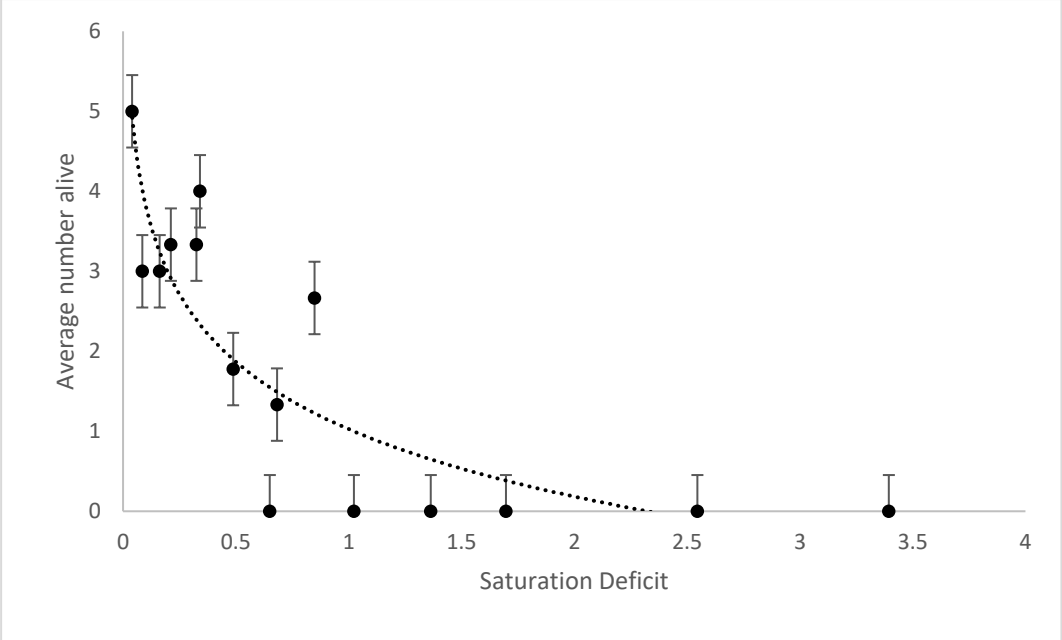


**Fig. 2.11** The average number of ticks alive on day 50 and the corresponding saturation deficit of the jar in which the ticks were kept, the saturation deficit being an integration of the humidity and temperature and defined as the drying power of the air ($Y=-1.271\ln(X)+1.0939$, $Z_{44}=5.8$, $P<0.001$, $r^2=0.6179$). The error bars represent standard error of the mean.

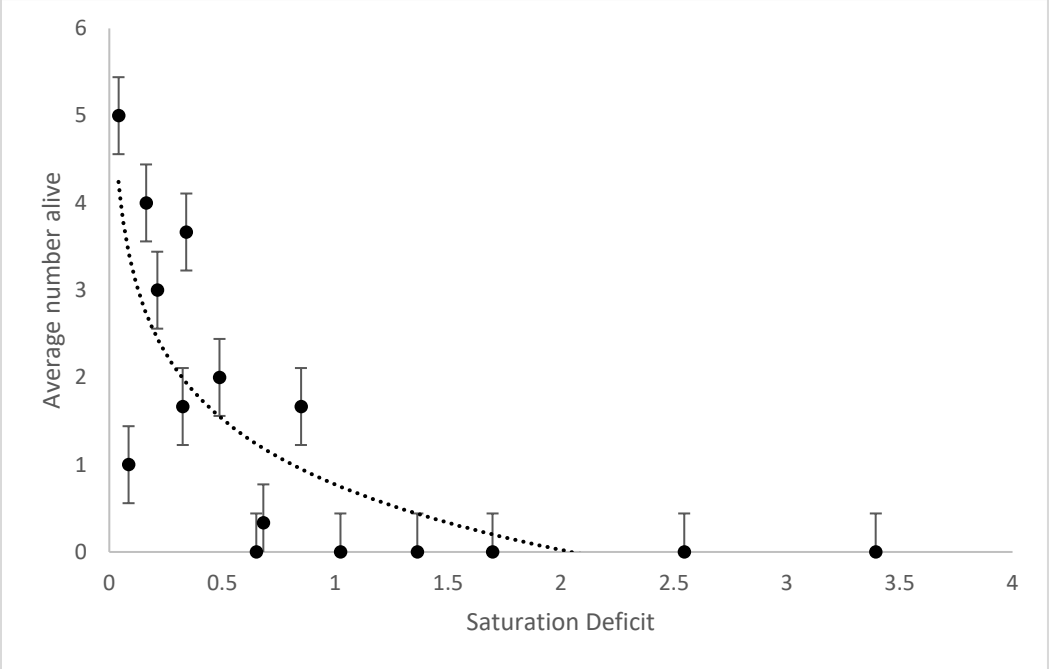


**Fig. 2.12** The average number of ticks alive on day 63 and the corresponding saturation deficit of the jar in which the ticks were kept, the saturation deficit being an integration of the humidity and temperature and defined as the drying power of the air deficits ($Y=-1.083\ln(X) + 0.7739$, $Z_{44}=5.4$, $P<0.001$, $r^2= 0.4874$). The error bars represent standard error of the mean.

## 2.4 Discussion

This study aimed to investigate the relationship between microclimatic factors, temperature and humidity, and the survival rate of *D. reticulatus* in the UK. Overall, the results indicate that the effect of humidity on tick survival was dependent upon the temperature and length of time the ticks were exposed to the conditions, specifically, the ticks were more likely to die when the temperature was higher, the humidity was lower, and the longer they had been exposed to these conditions in the jar. The ticks were not significantly affected by humidity until day 20 at 30°C, but not at 4°C and 15°C. By day 30, humidity significantly impacted tick survival at 30°C and 15°C, but not 4°C. By day 50, humidity significantly affected survival at all temperatures. In each instance, a lower humidity was associated with in a lower survival rate. The results indicate that the ticks were quicker to die at higher temperatures; as the time in which the ticks were in the jar increased, tick mortality was significantly higher in jars with higher temperatures first. By day 50, tick survival was affected by all temperatures.

Throughout, it is evident that both temperature and humidity act together to impact upon *D. reticulatus* survival. Hence, there is considerable value in using saturation deficit additionally to integrate these measures. In combining these variables, the single value of saturation deficit represents the drying power of the air, thus the overall effect of the conditions within each jar can be combined and the resultant survival rate studied in a simple way. Saturation deficit is also a more sensitive indicator of the moisture content of the air than humidity, as it considers the impact of temperature, undergoing greater variations for temperature change than relative humidity does (Anderson, 1936). Saturation deficit was not shown to have a significant impact upon tick survival rate until day 20. Over time, where the saturation deficit value was lower, the survival rate increased.

In previous literature, a study in eastern Poland found that the activity of adult *D. reticulatus* was not significantly impacted by relative humidity, only by temperature and photoperiod (Bartosik *et al.,* 2011). A variety of other studies observed a significant relationship between humidity and mortality or activity (Meyer-König *et al.,* 2001a; Buczek *et al.,* 2017). The results of this study support the latter observation.

It is not unexpected that at a lower saturation deficit, the mortality rate of *D. reticulatus* increased. At a higher saturation deficit, the difference between the moisture content in the air and in a tick is greater. As a result, the tick loses water via evaporation at a faster rate, and fatal desiccation is more likely to occur (Lees, 1946). In their natural environment in the UK, the abundance of *D. reticulatus* decreases over the summer months between June and August and this is thought to be due to higher temperatures and lower humidities (Tharme, 1993). This is expected, as these conditions would generate a greater saturation deficit. As discussed in Chapter 1, ticks have a variety of mechanisms to prevent water loss, such as the integument, spiracular valves to shield the respiratory system, and the excretion of nitrogenous waste in the form of guanine crystals. To rehydrate, ticks can drink water, absorb atmospheric vapour, and feed on a host (Needham and Teel, 1991; Meyer-König *et al.,* 2001b). However, during the study, the low humidities meant ticks were unable to absorb water efficiently from the air, creating a deficit. At higher humidities, the deficit was much smaller or non-existent, enabling ticks to retain their moisture content for a lot longer, resulting in a lower mortality rate. In their natural environment, ticks will actively move to more favourable areas of higher humidity which requires energy from fat reserves within the ticks (Mejlon and Jaenson, 1997; Randolph and Storey, 1999). Over the course of the experiments, when the jars were being checked within the first week or so, it was observed that ticks at 15°C and 30°C were already actively moving around their tubes prior to being taken out, whereas ticks being kept at 4°C only showed movement after having been out of the cooler conditions for 5 minutes or more. It is possible that at the higher temperatures the ticks increased their movement within the tubes, thus increasing their metabolic rate, in an attempt to move to an area more suited to their temperature and humidity requirements. As a result, the ticks' fat reserves were depleted much quicker than the fat reserves of the ticks within jars containing more favourable environments, where temperatures were lower and humidities were higher, and there was less movement and lower metabolic rates. This requires further investigation.

It has been suggested in previous studies that *D. reticulatus* is a 'psychrophilic' species, able to tolerate and thrive in low temperatures, whereas the tick species more common in the UK, *I. ricinus,* is 'mesophilic', preferring moderate temperatures (Hubálek *et al.,* 2003). In eastern and mainland Europe, *D. reticulatus* has been observed actively questing at air temperatures of 0.7°C

and 1°C and a soil temperature of -0.1°C whereas the threshold temperature of *I. ricinus* activity is thought to be 7°C (Nosek, 1972; Tharme, 1993; Perret *et al.,* 2000; Hubálek *et al.,* 2003). Some studies have not observed *I. ricinus* activity even at 7°C; one study, undertaken in Switzerland, reported that no questing activity was observed at temperatures under 10°C (Burri *et al.,* 2007) and an investigation into *I. ricinus* at 11 sites in Italy recorded the mean temperature at which nymphs and larva began questing as 10°C and 8°C respectively. A few ticks were also collected at temperatures as low as 3°C, but this was thought to be a result of some microhabitats being exposed to sun during the winter (Tagliapietra *et al.,* 2011). Another noted that questing *I. ricinus* ticks were only active when the temperature exceeded 5.2°C and only very rarely between 1.9°C and 3.8°C (Perret *et al.,* 2000). In this study, mortality was only observed in *D. reticulatus* at 4°C after 50 days, having been able to survive before this point even when the humidity was extremely low and without the vegetation layer which would be available in their natural environment to provide intermittent sanctuary from cold weather. This shows that *D. reticulatus* is able to survive comfortably in temperatures as low as 4°C and possibly lower, so supports the theory that *D. reticulatus* is able to withstand temperatures much lower than *I. ricinus,* both in the UK and eastern Europe. For confirmation, the experiments undertaken here could be repeated with *I. ricinus* ticks to allow comparison.

One of the most common tick species found on cats and dogs in the UK, second only to *I ricinus*, is *I. hexagonus*. Studies of *I. hexagonus* suggest that this species is active at even warmer temperatures than *I. ricinus* and *D. reticulatus,* and similarly high humidities. It is thought that I. *hexagonus* is more strongly associated with nests and burrows, habitats that are more insulated than the exposed, outside vegetation that *I. ricinus* and *D. reticulatus* use for questing (Arthur, 1951; Toutoungi *et al.,* 1993; Pfäffle *et al.,* 2011; Sherrard-Smith *et al.,* 2012).

While it is accepted that a high relative humidity is preferred by both *I. ricinus* and *D. reticulatus*, there are some contrasts in literature as to which relative humidity provides the equilibrium required to cease all water loss via evaporation from a tick. For *I. ricinus*, studies suggest that this is around 90% RH (Lees, 1946) and between 70- 80% RH (MacLeod, 1935). For *D. reticulatus*, a study in Poland observed the most activity when humidity was at its highest at the field site at around 55-65% RH (Buczek *et al.,* 2017). It is likely that ticks in this environment would have high rates of evaporation, and that frequent excursions into the vegetative layer beneath and

the use of the alternative rehydration strategies, as discussed in Chapter 1, would have helped to restore any water lost while questing. It is likely that these strategies were used as the results of this thesis suggest that moisture equilibrium is not reached even when ticks are in high humidity conditions at 80% RH. Ticks in tubes at 95% RH in 4°C temperatures all survived, however at 80% RH and 4°C throughout the experiments, ticks were steadily dying. This implies that at 80% RH, the equilibrium at which a tick no longer loses water to the surrounding air has not yet been reached. This research supports previous statements in literature based in mainland Europe that *D. reticulatus* requires relative humidities exceeding 80% for active water uptake from the atmosphere (MacLeod, 1935; Meyer-König *et al.,* 2001b).

# CHAPTER 3

## Discussion

Between 1880 to 2012, there was an average global warming of 0.65 to 1.06°C, combining both land and ocean surface temperature data (IPCC, 2014). It is thought that by 2035, relative to 1986-2005, the air temperature will increase by up to 0.7°C and by 2100, will exceed 1.5-2°C (IPCC, 2014). Beyond 2100, warming will continue even if anthropogenic emissions cease. If the mean global warming reaches 2°C, in the south east of the UK the temperature may increase another 3-4°C (based on the present day). The entirety of the UK would face minimum warming of 1- 2°C throughout the year. Cold winter days would be warmer by 1-1.5°C (Bernie *et al.,* 2018). Along with rising temperatures, there are other expected differences, such as changes in the level of precipitation, increased variability in climatic variables, and severe weather events. These climate changes are anticipated to alter emergence of vectors, and so vector-borne disease, in a variety of ways (Karbowiak, 2014; Ogden and Lindsay, 2016). These levels of climate warming are expected to have a variety of effects on ticks and, notably, on aspects of their behaviour, disease transmission and host responses, depending upon the tick species in question, the location at which populations are established, and its environmental requirements. There are a multitude of variables interacting dynamically, meaning changes to these systems are difficult to accurately predict (Dantas-Torres, 2015). However, the results from this thesis allow some scope for speculation as to how *D. reticulatus* distribution and abundance might change in the UK in the future, and how tick-borne disease might be impacted as a result.

An overall increase in temperature would be likely to make tick development faster, increase activity and lower mortality in more northerly latitudes which were previously considered as inhospitable, and reduce activity and increase mortality where temperature now exceeds the favourable threshold (Ogden and Lindsay, 2016). For instance, in South Africa, it is estimated that an increase in temperature of 2°C will be damaging for habitat suitability for four of the most problematic tick species, due to the unfavourable new high temperature (Estrada-Peña, 2003). As the climate becomes warmer in temperate zones, such as the UK, tick distributions are likely to move northward to escape the new hot climate and seek favourable temperatures in locations

previously too cold for survival during the winter months (Ogden and Lindsay, 2016). As the air warms, the saturation deficit between ticks and the atmosphere would be expected to increase, heightening the risk of desiccation, and forcing the tick distributions to move north. Additionally, rising temperatures will alter the seasonal activity patterns, such that activity is reduced in the summer months, but allowing them to be more active throughout the winter. This would promote the early questing activity of immature instars, encouraging population growth and dispersion (Estrada-Peña, 2015). *Dermacentor reticulatus* may be among those tick species which benefit the most, as the warmer climate would allow completion of the life cycle within a single year (Gray *et al.,* 2009; Jameson and Medlock, 2011).

The results presented in this thesis imply that *D. reticulatus* thrives in very cool environments, possibly because of the risks from desiccation. Therefore, as the climate warms due to increased anthropogenic activities, it might be that the distribution of *D. reticulatus* will spread further northwards. Ticks such as *I. ricinus,* and especially *I. hexagonus,* which tolerate warmer environments, as discussed in Chapter 2, may display a slower dispersion northwards as they would be better adapted and may thrive in the warmer temperatures generated through climate change in the south of the UK.

It has been suggested that a warmer climate across the UK may result in more successful incubation of pathogens within tick vectors (Medlock *et al.,* 2015). Lyme disease spirochetes regulate surface proteins during tick feeding to increase infectivity for the host, and it is thought that higher temperatures cause enhancement of infection through upregulation of these proteins (Obonyo *et al.,* 1999). Environmental temperature has been found to affect the vector competence of *Ixodes dammini*, for *Borrelia burgdorferi*, with temperatures of up to 27°C deemed adequate for transmission (Shih *et al.,* 1995). It is possible, therefore, that pathogens spread by *D. reticulatus* will also benefit from the warming climate and increasingly effective transmission between ticks and hosts will be observed; this provides opportunity for further research. Hence, it is likely that the pathogens transmitted by *D. reticulatus* will also become more abundant and more widely established in areas of the UK where they were not previously found. New and previously exotic pathogens may establish and spread more rapidly in the UK, particularly pathogens such as *Rickettsia raoultii* and tick-borne encephalitis virus (Tijsse-Klasen *et al.,* 2011; Tijsse-Klasen *et al.,* 2013). As a consequence of more successful incubation and transmission of pathogens and their

associated diseases, along with the widening distribution of *D. reticulatus*, the future incidence of tick-borne diseases is expected to increase.

Alternatively, there is some evidence that ticks can adapt rapidly to a warmer environment. Thus, the distribution of ticks may not change as much as expected during global warming. Populations of *I. ricinus* collected from a cline of climates using a latitudinal gradient (Scotland, Wales, Southern England, and France) successfully adapted their behaviour in response to the different temperatures in each location. Predictions suggest that they could advance their period of questing by a month under climate change (Gilbert *et al.,* 2014). It is widely accepted that the general threshold temperature for *I. ricinus* activity is 7°C and that a relative humidity exceeding 70-80% is required for survival (MacLeod, 1935; Tharme, 1993; Perret *et al.,* 2000). However, one study observed *I. ricinus* questing at 2.5°C in the Czech Republic and at a relative humidity of 45% (Hubálek *et al.,* 2003). *Dermacentor reticulatus* is present over a large geographical area, over which the climate varies substantially. It is therefore possible that *D. reticulatus* possesses a similar ability to adapt to conditions to maximize survival in each local climate. It is known that small populations of *D. reticulatus* have been present in the UK for over 100 years, however the time frame could be considerably longer (Medlock *et al.,* 2011). Therefore, there is a possibility that populations of *D. reticulatus* in the UK could be genetically distinct from one another and/or collectively genetically distinct from those on mainland Europe. There are currently no published studies on the subject. If this were the case, these populations could have considerable differences in environmental constraints, such as a differing tolerance and sensitivity to humidity or temperature due to varying adaptabilities. This presents an opportunity for future research into *D. reticulatus* climatic adaptation throughout the UK and Europe, gaining a valuable insight into how this species may disperse as the global climate warms. Additionally, while we know of 4 sites at which *D. reticulatus* can be found in the UK, there are potentially many more in sand dune systems or other historic rabbit warrens. In order to fully understand the future spread, it is vital to investigate the current distribution in more detail.

The effect of a warmer climate on hosts should also be considered, as ticks can only establish where there are suitable hosts for development and feeding. Some hosts may move northwards as the climate becomes warmer and more favourable, causing the dispersion of feeding ticks and the pathogens they carry at the same time (Jenson *et al.,* 2000). This presents

opportunity for future work to investigate the availability of wild hosts of *D. reticulatus* in the UK (small mammals such as rabbits and voles for larvae and nymphs and larger animals such as deer for adults), which may change their abundance and distribution as temperatures rise (Nosek, 1972).

This thesis has provided valuable information on the effect of microclimatic factors on the mortality of *D. reticulatus* in the UK. This information was previously lacking in literature. It is clear that both temperature and relative humidity have a significant effect on mortality and that when integrated into a measure of saturation deficit, where temperature is high and humidity is low, mortality increases significantly, presumably as a result of desiccation. These results suggest that *D. reticulatus* is a 'psychrophilic' species, able to endure colder temperatures than other UK ticks, such as *I. ricinus* and *I. hexagonus,* and requires a moist atmosphere surpassing 80% RH in order to cease water loss via evaporation. These conclusions allow for some speculation as to how *D. reticulatus* distribution and abundance might change under climate change along with the distribution of the pathogens they transmit, presenting a serious health risk for humans and domestic animals in the UK.

The primary limitation of this study was the closure of labs and cessation of fieldwork following the lockdown due to the Covid-19 pandemic. As a result, mortality could not be studied at a greater range of temperatures. Future work should include investigating mortality at lower temperatures, particularly the lower lethal temperature and the effects of cold shock.

Due to constraints, primarily due to the pandemic situation, and the limited availability of time to visit each location where ticks were collected, the ticks from all of the locations were mixed together in the jars, providing insights into the mortality of *D. reticulatus* under differing microclimatic conditions in the UK as a whole. The climate of each collection location differs, as described in Chapter2, and will undoubtedly have an effect on the temperature and humidity conditions that the ticks experience, both while questing and when rehydrating in vegetation. It is important to take this into consideration. In the future, a more sensitive study could be undertaken based on geographic variation using a different approach whereby ticks are separated according to the location they are collected from, allowing the results to be compared between populations in the UK. In this instance, it was not possible to collect a large enough sample size from each location in the limited time frame given to conduct the experiments to be able to meaningfully

compare populations. It might be that ticks have local adaptations to the climate in each different location in the UK.

While this thesis has explored the impact of temperature and humidity on the mortality rate of *D. reticulatus*, other environmental factors, such as soil temperature, photoperiod, vegetation type and cover, the abundance of host population and wind speed are also known to affect activity and mortality rate of ticks (Tharme, 1993; Bartosik *et al.,* 2011; Estrada-Peña, 2015). These variables also require investigation. Using the results presented here, along with further studies, modelling could be undertaken to see more specifically how climate change might alter the distribution and abundance of *D. reticulatus* in the UK in the near future. This has been undertaken for *I. ricinus* in the UK (Hancock *et al.,* 2011). It was concluded that the effect of temperature variation on *Ixodes ricinus* was dependent upon the cumulative annual temperature, and so will vary in locations across the UK. It might be that *D. reticulatus* modelling would show an analogous trend. It is important to conduct similar studies on *D. reticulatus* in the UK in order to understand how populations will change and how the risk of tick-borne disease could increase in the future.

# REFERENCES


Abdullah, S., Helps, C., Tasker, S., Newbury, H. and Wall, R. (2016). Ticks infesting domestic dogs in the UK: A large-scale surveillance programme. *Parasites and Vectors*, **9**, 391.

Abdullah, S., Helps, C., Tasker, S., Newbury, H. and Wall, R. (2018). Prevalence and distribution of *Borrelia* and *Babesia* species in ticks feeding on dogs in the U.K. *Medical and Veterinary Entomology*, **32**, 14–22.

Anderson, D. B. (1936). Relative humidity or vapor pressure deficit. *Ecology,* **17**, 277-282.

Arthur, D. R. (1951). The bionomics of *Ixodes hexagonus* Leach in Britain. *Parasitology*, **41**, 82–90.

Barker, S. C. and Murrell, A. (2004). Systematics and evolution of ticks with a list of valid genus and species names. *Parasitology*, **129**, 15-36.

Bartosik, K., Wiśniowski, Ł. and Buczek, A. (2011). Abundance and seasonal activity of adult *Dermacentor reticulatus* (Acari: Amblyommidae) in eastern Poland in relation to meteorological conditions and the photoperiod. *Annals of Agricultural and Environmental Medicine,* **18**, 340-344.

Bernie, D., Gohar, G., Good, P. and Lowe, J. A. (2018). UKCP18 Applied Projections of Future Climate over the UK, Met Office. https://www.metoffice.gov.uk/binaries/content/assets/metofficegovuk/pdf/research/ukcp/ukcp18-fact-sheet-derived-projections.pdf. Accessed 22 May 2020.

Biernat, B., Karbowiak, G., Werszko, J. and Stańczak, J. (2014). Prevalence of tick-borne encephalitis virus (TBEV) RNA in *Dermacentor reticulatus* ticks from natural and urban environment, Poland. *Experimental and Applied Acarology*, **64**, 543–551.

Boehnke, D., Gebhardt, R., Petney, T. and Norra, S. (2017). On the complexity of measuring forests microclimate and interpreting its relevance in habitat ecology: The example of *Ixodes ricinus* ticks. *Parasites and Vectors*, **10**.

Bremer, W., Schaefer, J., Wagner, R., Ewing, S., Rikihisa, Y., Glen, R., Needham, G., Jittapalapong, S., Moore, D. and Stich, R. (2005). Transstadial and intrastadial experimental transmission of *Ehrlichia canis* by male *Rhipicephalus sanguineus*. *Veterinary Parasitology,* **131**, 95-105.

Buczek, A., Bartosik, K., Zając, Z. and Stanko, M. (2015). Host-feeding behaviour of *Dermacentor reticulatus* and *Dermacentor marginatus* in mono-specific and inter-specific infestations. *Parasites & Vectors*, **8**, 470.

Buczek, A., Zając, Z., Woźniak, A., Kulina, D. and Bartosik, K. (2017). Locomotor activity of adult *Dermacentor reticulatus* ticks (Ixodida: Ixodidae) in natural conditions. *Annals of Agricultural and Environmental Medicine*, **24**, 271–275.

Bullová, E., Lukáň, M., Stanko, M. and Peťko, B. (2009). Spatial distribution of *Dermacentor reticulatus* tick in Slovakia in the beginning of the 21st century. *Veterinary Parasitology*, **165**, 357–360.

Burri, C., Cadenas, F. M., Douet, V., Moret, J. and Gern, L. (2007). *Ixodes ricinus* density and infection prevalence of *Borrelia burgdorferi* sensu lato along a north-facing altitudinal gradient in the Rhône Valley (Switzerland). *Vector-Borne and Zoonotic Diseases*, **7**, 50–58.

Camicas, J. L., Hervy, J. P., Adam, F. and Morel, P. C. (1998). The ticks of the world (Acarida, Ixodida): nomenclature, described stages, hosts, distribution. *Éditions de l'ORSTOM,* 233.

Cerny, V., Szymanski, S., Dusbabek, F., Daniel, M. and Honzakova, E. (1982). Survival of unfed *Dermacentor reticulatus* (Fabr.) adults under natural conditions. *Wiadomosci Parazytologiczne*, **28,** 27–31.

Charrier, N. P., Hermouet, A., Hervet, C., Agoulon, A., Barker, S. C., Heylen, D., Toty, C., McCoy, K., Plantard, O. and Rispe, C. (2019). A transcriptome-based phylogenetic study of hard ticks (Ixodidae). *Scientific Reports*, **9**.

Coultous, R. M., Phipps, P., Dalley, C., Lewis, J., Hammond, T. A., Shiels, B. R. Weir, W. and Sutton, D. G. M. (2019). Equine piroplasmosis status in the UK: An assessment of laboratory diagnostic submissions and techniques. *Veterinary Record*, **184**, 95.

Dantas-Torres, F. (2015). Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. *International Journal for Parasitology: Parasites and Wildlife*, **4**, 452–461.

Dautel, H., Dippel, C., Oehme, R., Hartelt, K. and Schettler, E. (2006). Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of Rickettsia sp. RpA4. *International Journal of Medical Microbiology*, **296**, 149–156.

de Marco, M. del M. F., Hernández-Triana, L. M., Phipps, L. P., Hansford, K., Mitchell, E. S., Cull, B., Swainsbury, C., Fooks, A., Medlock, J. and Johnson, N. (2017). Emergence of *Babesia canis* in southern England. *Parasites and Vectors*, **10**, 241.

Dusbábek, F. (1996). Nymphal sexual dimorphism in the sheep tick *Ixodes ricinus* (Acari: Ixodidae). *Folia Parasitologica*, **43**, 75–79.

Edney, E. B. (1977). *Zoophysiology and Ecology: Water balance in land arthropods*. Springer-Verlag Publishing, Berlin, Germany.

Estrada-Peña, A. (2003). Climate change decreases habitat suitability for some tick species (Acari: Ixodidae) in South Africa. *Onderstepoort Journal of Veterinary Research*, **70**, 79–93.

Estrada-Peña, A. (2015). Ticks as vectors: Taxonomy, biology and ecology. *OIE Revue Scientifique et Technique*, **34**, 53–65.

Estrada-Peña, A., Gray, J. S., Kahl, O., Lane, R. S. and Nijhof, A. M. (2013). Research on the ecology of ticks and tick-borne pathogens-methodological principles and caveats. *Frontiers in Cellular and Infection Microbiology*, **4**.

European Centre for Disease Prevention and Control and European Food Safety Authority (ECDC and EFSA) (2019). https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/tick-maps. Accessed 31 January 2020.

Evans, G. O. (1951). The distribution and economic Importance of *Ixodes ricinus* in Wales and the Welsh Border Counties with special reference to N.W. Cardiganshire. *Bulletin of Entomological Research*, **41**, 469–485.

Feldman-Muhsam, B. and Borut, S. (1971). Copulation in Ixodid ticks. *The Journal of Parasitology*, **57**, 630.

Földvári, G. and Farkas, R. (2005). Ixodid tick species attaching to dogs in Hungary. *Veterinary Parasitology*, **129**, 125–131.

Földvári, G., Rigó, K. and Lakos, A. (2013). Transmission of *Rickettsia slovaca* and *Rickettsia raoultii* by male *Dermacentor marginatus* and *Dermacentor reticulatus* ticks to humans. *Diagnostic Microbiology and Infectious Disease*, **76**, 387–389.

Földvári, G., Široký, P., Szekeres, S., Majoros, G. and Sprong, H. (2016). *Dermacentor reticulatus*: A vector on the rise. *Parasites and Vectors*, **9**.

Gale, P., Stephenson, B., Brouwer, A., Martinez, M., de la Torre, A., Bosch, J., Foley-Fisher, M., Bonilauri, P., Lindström, A., de Vos, C., Scremin, M., Liu, Z., Kelly, L. and Muñoz, M. J. (2012). Impact of climate change on risk of incursion of Crimean-Congo haemorrhagic fever virus in livestock in Europe through migratory birds. *Journal of Applied Microbiology*, **112**, 246–257.

Gerstengarbe, F. W. and Werner, P. C. (2008). Climate development in the last century - Global and regional. *International Journal of Medical Microbiology*, **298**, 5–11.

Gilbert, L., Aungier, J. and Tomkins, J. L. (2014). Climate of origin affects tick (*Ixodes ricinus*) host-seeking behavior in response to temperature: Implications for resilience to climate change? *Ecology and Evolution*, **4**, 1186–1198.

Graham, R. I., Mainwaring, M. C. and Du Feu, R. (2010). Detection of spotted fever group *Rickettsia* spp. from bird ticks in the U.K. *Medical and Veterinary Entomology*, **24**, 340–343.

Gray, J. S., Dautel, H., Estrada-Peña, A., Kahl, O. and Lindgren, E. (2009). Effects of climate change on ticks and tick-borne diseases in Europe. *Interdisciplinary Perspectives on Infectious Diseases*, 1-12.

Gray, J. S., Estrada-Peña, A. and Zintl, A. (2019). Vectors of *Babesiosis*. *Annual Review of Entomology*, **64**, 149–165.

Gray, J. S., Kahl, O., Lane, R. S., Levin, M. L. and Tsao, J. I. (2016). Diapause in ticks of the medically important *Ixodes ricinus* species complex. *Ticks and Tick-Borne Diseases*, **7**, 992–1003.

Grist, N. (1992). Ticks are topical. *Journal of Infection*, **24**, 117–121.

Gritsun, T. S., Lashkevich, V. A. and Gould, E. A. (2003). Tick-borne encephalitis. *Antiviral Research*, **57**, 129–146.

Guglielmone, A. A., Robbins, R. G., Apanaskevich, D. A., Petney, T. N., Estrada-Peňa, A., Horak, I. G., Shao, R. and Barker, S. C. (2010). The *Argasidae*, *Ixodidae* and *Nuttalliellidae (*Acari: Ixodida) of the world: A list of valid species names. *Zootaxa*, **2528**, 1–28.

Guidi, E., Pradier, S., Lebert, I. and Leblond, A. (2015). Piroplasmosis in an endemic area: analysis of the risk factors and their implications in the control of Theileriosis and Babesiosis in horses. *Parasitology Research*, **114**, 71–83.

Hancock, P. A., Brackley, R. and Palmer, S. C. F. (2011). Modelling the effect of temperature variation on the seasonal dynamics of *Ixodes ricinus* tick populations. *International Journal for Parasitology*, **41**, 513–522.

Harris, S. and Thompson, G. B. (1978). Populations of the ticks *Ixodes (Pholeoixodes) hexagonus* and *Ixodes (Pholeoixodes) canisuga* infesting suburban foxes, *Vulpes vulpes*. *Journal of Zoology*, **186**, 83–93.

Hoogstraal, H. (1967). Ticks in relation to human diseases caused by *Rickettsia* species. *Annual Review of Entomology*, **12**, 377–420.

Hoogstraal, H. (1985). Argasid and Nuttalliellid ticks as parasites and vectors. *Advances in Parasitology*, **24**, 135–238.

Hornok, S., Sándor, A. D., Beck, R., Farkas, R., Beati, L., Kontschán, J., Takács, N., Földvári, G., Silaghi, C., Meyer-Kayser, E., Hodžić, A., Tomanović, S., Abdullah, S., Wall, R., Estrada-Peña, A., Duscher, G. and Plantard, O. (2017). Contributions to the phylogeny of *Ixodes (Pholeoixodes)*

*canisuga, I. (Ph.) kaiseri, I. (Ph.) hexagonus* and a simple pictorial key for the identification of their females. *Parasites and Vectors*. 10.

Hovius, J. W. R. (2009). Spitting image: Tick saliva assists the causative agent of Lyme disease in evading host skins innate immune response. *Journal of Investigative Dermatology*, 129, 2337–2339.

Hubálek, Z., Halouzka, J. and Juricová, Z. (2003). Host-seeking activity of ixodid ticks in relation to weather variables. *Journal of Vector Ecology: Journal of the Society for Vector Ecology*, 28, 159–165.

IPCC. (2014). *Climate Change 2014: Synthesis Report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* 58-60. https://www.ipcc.ch/site/assets/uploads/2018/02/SYR_AR5_FINAL_full.pdf. Accessed 22 May 2020.

Irwin, P. J. (2009). Canine babesiosis: From molecular taxonomy to control. *Parasites and Vectors*, 2, S4.

Jameson, L. J. and Medlock, J. M. (2011). Tick surveillance in Great Britain. *Vector-Borne and Zoonotic Diseases*, 11, 403–412.

Jongejan, F. and Uilenberg, G. (2004). The global importance of ticks. *Parasitology*, 129, 3-14.

Karbowiak, G. (2014). The occurrence of the *Dermacentor reticulatus* tick-its expansion to new areas and possible causes. *Annals of Parasitology*, 60, 37–47.

Karbowiak, G., Izdebska, J. N., Czaplińska, U. and Wita, I. (2003). Cases of survival of the winter by Ixodidae ticks on the hosts in the Białowieża Primeval Forest. *Międzynarodowe Sympozjum Stawonogi Pasożytnicze, Alergogenne i Jadowite - Znaczenie Medyczne i Sanitarne,* 77-82.

Kaufman, R. (1989). Tick-host interaction: A synthesis of current concepts. *Parasitology Today*, 5, 47–56.

Kiszewski, A. E., Matuschka, F.-R. and Spielman, A. (2001). Mating Strategies in Ixodid Ticks. *Annual Review Entomology*, 46, 167-82.

Kubes, M., Fuchsberger, N., Labuda, M., Zuffová, E. and Nuttall, P. A. (1994). Salivary gland extracts of partially fed *Dermacentor reticulatus* ticks decrease natural killer cell activity in vitro. *Immunology*, 82, 113–116.lee

Lees, A. D. (1946). The water balance in *Ixodes ricinus* L. and certain other species of ticks. *Parasitology*, 37, 1–20.

Lejal, E., Marsot, M., Chalvet-Monfray, K., Cosson, J. F., Moutailler, S., Vayssier-Taussat, M. and Pollet, T. (2019). A three-years assessment of *Ixodes ricinus*-borne pathogens in a French peri-urban forest. *Parasites and Vectors*, **12**.

MacLeod, J. (1935). *Ixodes ricinus* in relation to its physical environment: II. The factors governing survival and activity. *Parasitology*, **27**, 123–144.

Mans, B. J., de Klerk, D., Pienaar, R. and Latif, A. A. (2011). *Nuttalliella namaqua*: A living fossil and closest relative to the ancestral tick lineage: Implications for the evolution of blood-feeding in ticks. *PLoS ONE*, **6**.

Marchal, C., Schramm, F., Kern, A., Luft, B. J., Yang, X., Schuijt, T., Hovius, J., Jaulhac, B. and Boulanger, N. (2011). Antialarmin effect of tick saliva during the transmission of lyme disease. *Infection and Immunity*, **79**, 774–785.

Martinod, S. and Gilot, B. (1991). Epidemiology of canine babesiosis in relation to the activity of *Dermacentor reticulatus* in southern Jura (France). *Experimental & Applied Acarology*, **11**, 215–222.

Matjila, T. P., Nijhof, A. M., Taoufik, A., Houwers, D., Teske, E., Penzhorn, B. L., de Lange, T. and Jongejan, F. (2005). Autochthonous canine babesiosis in the Netherlands. *Veterinary Parasitology*, **131**, 23–29.

McGarry, J. W. (2011). Travel and disease vector ticks. *Travel Medicine and Infectious Disease*, **9**, 49–59.

Medlock, J. M., Hansford, K. M., Vaux, A. G. C., Cull, B., Abdullah, S., Pietzsch, M. E., Wall,R., Johnson, N. and Phipps, L. P. (2017). Distribution of the tick *Dermacentor reticulatus* in the United Kingdom. *Medical and Veterinary Entomology*, **31**, 281–288.

Medlock, J. M., Hansford, K., Vaux, A., Cull, B., Gillingham, E. and Leach, S. (2018). Assessment of the public health threats posed by vector-borne disease in the United Kingdom (UK). *Journal of Environmental Research and Public Health*, **15**.

Medlock, J. M., Jameson, L. J. and Phipps, L. P. (2011) Status of *Dermacentor reticulatus* in the UK. *Veterinary Record,* **168,** 386-387.

Medlock, J. M. and Leach, S. A. (2015). Effect of climate change on vector-borne disease risk in the UK. *The Lancet Infectious Diseases*, **15**, 721–730.

Mejlon, H. A. and Jaenson, T. G. T. (1997). Questing behaviour of *Ixodes ricinus* ticks (Acari: Ixodidae). *Experimental and Applied Acarology*, **21**, 747–754.

Menn, B. (2006). Untersuchungen zur Verbreitung und Ökologie von *Dermacentor* spec. *(*Ixodidae, Acari) in Deutschland. PhD Thesis: *Institute of Medical Microbiology, Immunology and Parasitology,* 80.

Met Office, UK (2020). Summaries of the climate characteristics of 11 regions of the UK. https://www.metoffice.gov.uk/research/climate/maps-and-data/regional-climates/index. Accessed 25 November 2020.

Meyer-König, A., Zahler, M. and Gothe, R. (2001a). Studies on survival and water balance of unfed adult *Dermacentor marginatus* and *D. reticulatus* ticks (Acari: Ixodidae). *Experimental and Applied Acarology*, **25**, 993–1004.

Meyer-König, A., Zahler, M. and Gothe, R. (2001b). Studies on the critical water mass and the rehydration potential of unfed adult *Dermacentor marginatus* and *D. reticulatus* ticks (Acari: Ixodidae). *Experimental and Applied Acarology*, **25**, 505–516.

Mierzejewska, E. J., Alsarraf, M., Behnke, J. M. and Bajer, A. (2015a). The effect of changes in agricultural practices on the density of *Dermacentor reticulatus* ticks. *Veterinary Parasitology*, **211**, 259–265.

Mierzejewska, E. J., Estrada-Peña, A., Alsarraf, M., Kowalec, M. and Bajer, A. (2016). Mapping of *Dermacentor reticulatus* expansion in Poland in 2012-2014. *Ticks and Tick-Borne Diseases*, **7**, 94–106.

Mierzejewska, E. J., Welc-Falęciak, R., Bednarska, M., Rodo, A. and Bajer, A. (2014). The first evidence for vertical transmission of *Babesia canis* in a litter of Central Asian Shepherd dogs. *Annals of Agricultural and Environmental Medicine*, **21**, 500–503.

Mierzejewska, E. J., Welc-Faleciak, R., Karbowiak, G., Kowalec, M., Behnke, J. M. and Bajer, A. (2015b). Dominance of *Dermacentor reticulatus* over *Ixodes ricinus* (Ixodidae) on livestock, companion animals and wild ruminants in eastern and central Poland. *Experimental and Applied Acarology*, **66**, 83–101.

Needham, G. and Teel, P. (1991). Off-host physiological ecology of Ixodid ticks. *Annual Review of Entomology*, **36**, 659–681.

Nosek, J. (1972). The ecology and public health importance of *Dermacentor marginatus* and *D. reticulatus* ticks in Central Europe. *Folia Parasitologica*, **19**, 93–102.

Nuttall, P. A. (2019). Tick saliva and its role in pathogen transmission. *Weiner klinische Wochenschrift*. 1-12.

Obonyo, M., Munderloh, U. G., Fingerle, V., Wilske, B. and Kurtti, T. J. (1999). *Borrelia burgdorferi* in tick cell culture modulates expression of outer surface proteins A and C in response to temperature. *Journal of Clinical Microbiology*, **37**, 2137–2141.

Ogden, N. H., Cripps, P., Davison, C. C., Owen, G., Parry, J. M., Timms, B. J. and Forbes, A. B. (2000). The ixodid tick species attaching to domestic dogs and cats in Great Britain and Ireland. *Medical and Veterinary Entomology*, **14**, 332-338.

Ogden, N. H. and Lindsay, L. R. (2016). Effects of climate and climate change on vectors and vector-borne diseases: ticks are different. *Trends in Parasitology*, **32**, 646–656.

Oliver, J. H. (1989). Biology and systematics of ticks (Acari: Ixodida). *Annual Review of Ecology and Systematics,* **20**, 397–430.

Oliver, J. H., Al-Ahmadi, Z. and Osburn, R. L. (1974). Reproduction in ticks (Acari: Ixodoidea). 3. Copulation in *Dermacentor occidentalis* marx and *Haemaphysalis leporispalustris* (Packard) (Ixodidae). *The Journal of Parasitology*, **60**, 499.

Olivieri, E., Gazzonis, A. L., Zanzani, S. A., Veronesi, F. and Manfredi, M. T. (2017). Seasonal dynamics of adult *Dermacentor reticulatus* in a peri-urban park in southern Europe. *Ticks and Tick-Borne Diseases*, **8**, 772–779.

Paulauskas, A., Galdikas, M., Galdikaitė-Brazienė, E., Stanko, M., Kahl, O., Karbowiak, G. and Radzijevskaja, J. (2018). Microsatellite-based genetic diversity of *Dermacentor reticulatus* in Europe. *Infection, Genetics and Evolution*, **66**, 200–209.

Perret, J. L., Guigoz, E., Rais, O. and Gern, L. (2000). Influence of saturation deficit and temperature on *Ixodes ricinus* tick questing activity in a Lyme borreliosis-endemic area (Switzerland). *Parasitology Research*, **86**, 554–557.

Perret, J. L., Guerin, P. M., Diehl, P. A., Vlimant, M. and Gern, L. (2003). Darkness induces mobility, and saturation deficit limits questing duration, in the tick *Ixodes ricinus*. *Journal of Experimental Biology*, **206**, 1809–1815.

Petney, T. N. and Bull, C. M. (1981). A non-specific aggregation pheromone in two Australian reptile ticks. *Animal Behaviour*, **29**, 181–185.

Pfäffle, M., Littwin, N. and Petney, T. (2015). Host preferences of immature *Dermacentor reticulatus* (Acari: Ixodidae) in a forest habitat in Germany. *Ticks and Tick-Borne Diseases*, **6**, 508–515.

Pfäffle, M., Petney, T., Skuballa, J. and Taraschewski, H. (2011). Comparative population dynamics of a generalist (*Ixodes ricinus*) and specialist tick *(I. hexagonus*) species from European hedgehogs. *Experimental and Applied Acarology*, **54**, 151–164.

Pietzsch, M. E., Hansford, K. M., Cull, B., Jahfari, S., Sprong, H. and Medlock, J. M. (2015). Detection of *Dermacentor marginatus* and a possible *Rickettsia slovaca* case in the United Kingdom - The risk of the visiting traveller. *Travel Medicine and Infectious Disease*, **13**, 200–201.

Pietzsch, M. E., Medlock, J. M., Jones, L., Avenell, D., Abbott, J., Harding, P. and Leach, S. (2005). Distribution of *Ixodes ricinus* in the British Isles: Investigation of historical records. *Medical and Veterinary Entomology*, **19**, 306–314.

Public Health England (2019). *Tick-borne encephalitis virus detected in ticks in the UK.* https://www.gov.uk/government/news/tick-borne-encephalitis-virus-detected-in-ticks-in-the-uk. Accessed 31 January 2020.

Randolph, S. E. (2004a). Tick ecology: Processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology*, **129,** 37–65.

Randolph, S. E. (2004b). Evidence that climate change has caused "emergence" of tick-borne diseases in Europe? *International Journal of Medical Microbiology, Supplement*, **293**, 5–15.

Randolph, S. E. and Craine, N. G. (1995). General framework for comparative quantitative studies on transmission of tick-borne diseases using Lyme borreliosis in Europe as an example. *Journal of Medical Entomology*, **32**, 765–777.

Randolph, S. E., Green, R. M., Hoodless, A. N. and Peacey, M. F. (2002). An empirical quantitative framework for the seasonal population dynamics of the tick Ixodes ricinus. *International Journal for Parasitology*, **32**, 979–989.

Randolph, S. E., Miklisová, D., Lysy, J., Rogers, D. J. and Labuda, M. (1999). Incidence from coincidence: Patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. *Parasitology*, **118**, 177–186.

Randolph, S. E. and Storey, K. (1999). Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): Implications for parasite transmission. *Journal of Medical Entomology*, **36**, 741–748.

Razumova, I. V. (1998). The activity of *Dermacentor reticulatus* Fabr. (Ixodidae) ticks in nature. *Meditsinskaia Parazitologiia i Parazitarnye Bolezni*, 8–14.

Rubel, F., Brugger, K., Monazahian, M., Habedank, B., Dautel, H., Leverenz, S. and Kahl, O. (2014). The first German map of georeferenced ixodid tick locations. *Parasites and Vectors*, **7**, 477.

Rubel, F., Brugger, K., Pfeffer, M., Chitimia-Dobler, L., Didyk, Y. M., Leverenz, S., Dautel, H. and Kahl, O. (2016). Geographical distribution of *Dermacentor marginatus* and *Dermacentor reticulatus* in Europe. *Ticks and Tick-Borne Diseases*, **7**, 224–233.

Sampieri, B., Moreira, J. C. S., Páez, F. A. R. and Camargo-Mathias, M. (2016). Comparative morphology of the reproductive system and germ cells of Amblyomma ticks (Acari: Ixodidae): A contribution to Ixodidae systematics. *Journal of Microscopy and Ultrastructure*, **4**, 95.

Scoles, G. A. and Ueti, M. W. (2015). Vector ecology of Equine Piroplasmosis. *Annual Review of Entomology*, **60**, 561–580.

Sherrard-Smith, E., Chadwick, E. and Cable, J. (2012). Abiotic and biotic factors associated with tick population dynamics on a mammalian host: *Ixodes hexagonus* infesting otters, *Lutra lutra*. *PLoS ONE*, **7**.

Shih, C. M., Telford, S. R. and Spielman, A. (1995). Effect of ambient temperature on competence of deer ticks as hosts for Lyme disease spirochetes. *Journal of Clinical Microbiology*, **33**, 958–961.

Silva-Pinto, A., de Lurdes Santos, M. and Sarmento, A. (2014). Tick-borne lymphadenopathy, an emerging disease. *Ticks and Tick-Borne Diseases*, **6**, 656–659.

Šimo, L., Kocáková, P., Sláviková, M., Kubeš, M., Hajnická, V., Vančová, I. and Slovák, M. (2004). *Dermacentor reticulatus* female feeding in laboratory. *Biologia - Section Zoology*, **59**, 655–660.

Šimo, L., Kazimirova, M., Richardson, J. and Bonnet, S. I. (2017). The essential role of tick salivary glands and saliva in tick feeding and pathogen transmission. *Frontiers in Cellular and Infection Microbiology*, **7**, 281.

Solomon, M. E. (1951). Control of humidity with potassium hydroxide, sulphuric acid or other solutions. *Bulletin of Entomological Research,* **42**, 543-554.

Sonenshine, D. E. and Mather, T. N. (1994). *Ecological Dynamics of Tick-Borne Zoonoses.* Oxford University Press, 447.

Sonenshine, D. E. and Roe, R. M. (2014). *Biology of Ticks.* Oxford University Press, 61.

Špitalská, E., Štefanidesová, K., Kocianová, E. and Boldiš, V. (2012). *Rickettsia slovaca* and *Rickettsia raoultii* in *Dermacentor marginatus* and *Dermacentor reticulatus* ticks from Slovak Republic. *Experimental and Applied Acarology*, **57**, 189–197.

Sréter, T., Széll, Z. and Varga, I. (2005). Spatial distribution of *Dermacentor reticulatus* and *Ixodes ricinus* in Hungary: Evidence for change? *Veterinary Parasitology*, **128**, 347–351.

Süss, J., Klaus, C., Gerstengarbe, F. W. and Werner, P. C. (2008). What makes ticks tick? Climate change, ticks, and tick-borne diseases. *Journal of Travel Medicine*, **15**, 39–45.

Swainsbury, C., Bengtson, G. and Hill, P. (2016). Babesiosis in dogs. *The Veterinary Record,* **178**.

Tagliapietra, V., Rosà, R., Arnoldi, D., Cagnacci, F., Capelli, G., Montarsi, F., Hauffe, H. C. and Rizzoli, A. (2011). Saturation deficit and deer density affect questing activity and local abundance of *Ixodes ricinus* (Acari, Ixodidae) in Italy. *Veterinary Parasitology*, **183**, 114–124.

Tharme, AP. (1993). Ecological studies on the tick *Dermacentor reticulatus.* PhD Thesis, University of Wales.

Tijsse-Klasen, E., Hansford, K. M., Jahfari, S., Phipps, P., Sprong, H. and Medlock, J. M. (2013). Spotted fever group rickettsiae in *Dermacentor reticulatus* and *Haemaphysalis punctata* ticks in the UK. *Parasites and Vectors*, **6**.

Tijsse-Klasen, E., Jameson, L. J., Fonville, M., Leach, S., Sprong, H. and Medlock, J. M. (2011). First detection of spotted fever group rickettsiae in *Ixodes ricinus* and *Dermacentor reticulatus* ticks in the UK. *Epidemiology and Infection*, **139**, 524–529.

Tokhov, Y. M., Lutsuk, S. N. and Dyachenko, Y. V. (2014). Phenology of ixodid ticks of the genus *Dermacentor* in the Central Ciscaucasia. *Entomological Review*, **94**, 426–433.

Toutoungi, L. N., Gern, L. and Aeschlimann, A. (1993). Biology of *Ixodes* (Pholeoixodes) *hexagonus* under laboratory conditions. Part I. Immature stages. *Experimental and Applied Acarology*, **17**, 655–662.

Uspensky, I. (2002). Preliminary observations on specific adaptations of exophilic ixodid ticks to forests or open country habitats. *Experimental and Applied Acarology*, **28**, 147-154.

Walker, M. (2018a). The biology and ecology of the Sheep Tick *Ixodes ricinus*. *Antenna: The Royal Entomological Society,* **42**, 61-65.

Walker, M. (2018b). The hedgehog tick, *Ixodes hexagonus* (Leach, 1815) (Acari: Ixodidae); The natural history and ecology of a nest ectoparasite. *Systematic and Applied Acarology,* **23**, 680-714.

Wang, F., Wang, D., Guo, G., Hu, Y., Wei, J. and Liu, J. (2019). Species delimitation of the *Dermacentor* ticks based on phylogenetic clustering and niche modeling. *PeerJ*, **7**.

Wójcik-Fatla, A., Cisak, E., Zajac, V., Zwoliński, J. and Dutkiewicz, J. (2011). Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from the Lublin region (eastern Poland). *Ticks and Tick-Borne Diseases*, **2**, 16–19.

Zivkovic, Z., Nijhof, A. M., De La Fuente, J., Kocan, K. M. and Jongejan, F. (2007). Experimental transmission of anaplasma marginale by male *Dermacentor reticulatus. BMC Veterinary Research*, **3**, 32.

Zygner, W., Górski, P. and Wçdrychowicz, H. (2009). New localities of *Dermacentor reticulatus* tick (vector of *Babesia canis canis*) in central and eastern Poland. *Polish Journal of Veterinary Sciences*, **12**, 549–555.